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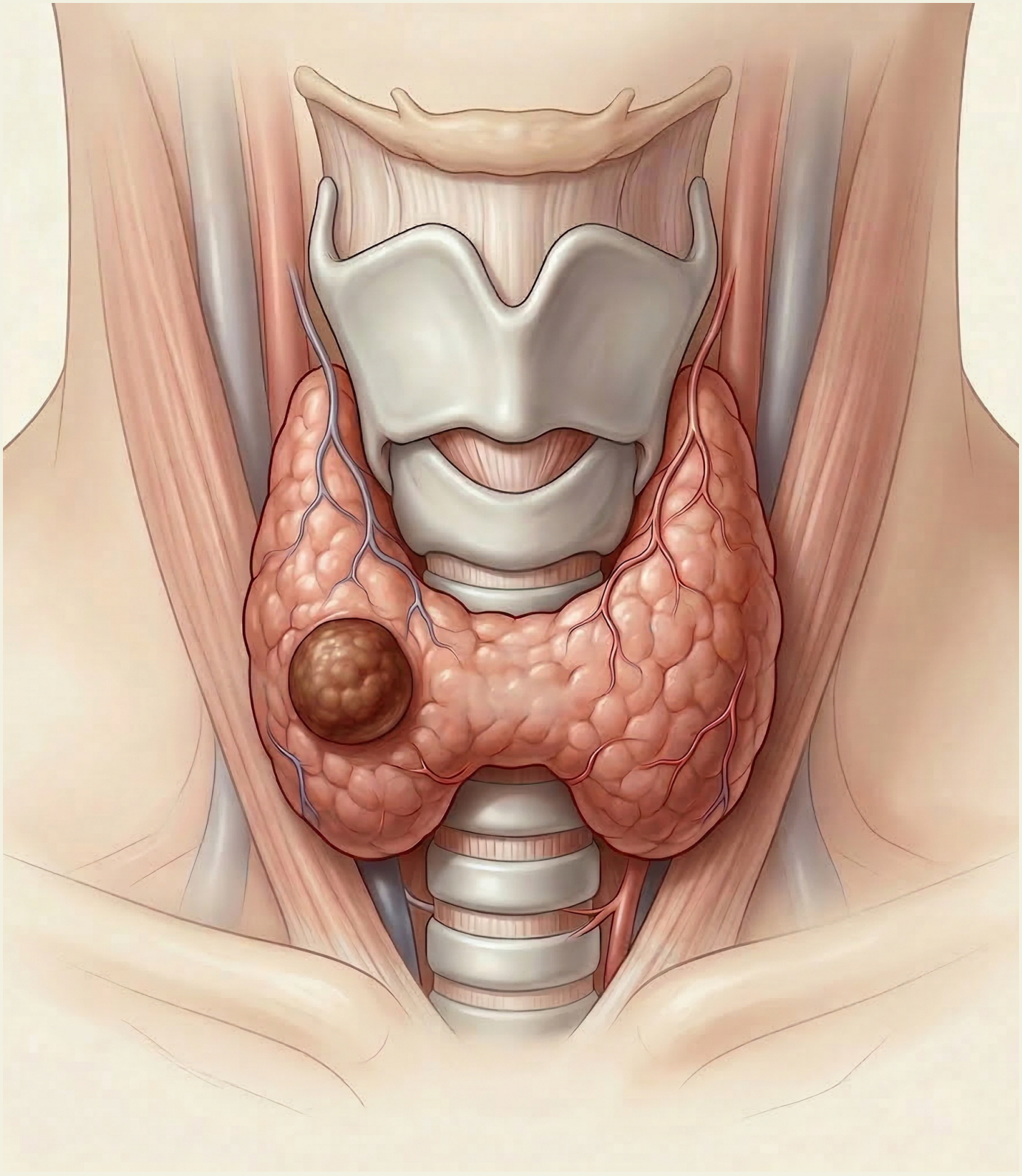

Thyroid Cancer Management: *Primum non nocere!*

EDITORIAL

THE DILEMMA OF INTERMEDIATE-RISK PAPILLARY THYROID CARCINOMA AND A POTENTIAL SOLUTION

One of the major topics of debate at the 10th International Thyroid Cancer Meeting, held in São Paulo last month, was the therapeutic dilemma posed by patients with intermediate-risk papillary thyroid carcinoma. This group, representing a substantial proportion of cases, if not the largest, has reported recurrence rates of up to 30%. Historically, clinicians have tended to favor overtreatment rather than undertreatment when managing patients within this risk category. Accordingly, total thyroidectomy followed by radioactive iodine therapy has long been considered the standard of care in such situations.

Following the publication of the 2025 American Thyroid Association (ATA) guidelines, the prevailing concept is to "do more" only for those who truly require it, namely, patients at the higher end of the intermediate-risk spectrum, whose recurrence risk is estimated at approximately 16% to 30%. This subgroup represents intermediate risk approaching high risk, without meeting formal criteria for high risk. It includes patients with tumors up to 4 cm in diameter presenting with bilateral tumor multifocality greater than 1 cm, or clinically evident lateral cervical lymph node metastases (N1b), all smaller than 3 cm, or the presence of two or more low-intermediate-risk features, as well as aggressive histological subtypes or vascular invasion. In such cases, total thyroidectomy is generally recommended to enable subsequent radioactive iodine (I-131) therapy, with the aim of reducing recurrence rates, which remain clinically meaningful in this subcategory.

The intent is to distinguish this subgroup from patients at the lower end of the intermediate-risk category, whose recurrence rates are approximately 10% to 15% and for whom partial thyroidectomy (lobectomy) may be suficiente, an instance where "less is more." Performing total thyroidectomy solely to facilitate radioactive iodine therapy would likely constitute overtreatment in this population, given its proximity to the low-risk category. This subgroup includes patients with tumors larger than 4 cm, or tumors smaller than 4 cm with unilateral multifocality, microscopic extrathyroidal extension, or more than five metastatic lymph nodes in the central compartment, all measuring between 2 mm and 3 cm. Negative surgical margins, or microscopic positivity limited to the posterior margin (i.e., the margin adjacent to the trachea or to structures within the recurrent laryngeal nerve territory), also characterize low-intermediate-risk disease. The guiding principle for this subgroup is treatment de-escalation, given the relatively low recurrence rates observed.

Over the years, the management of papillary thyroid carcinoma has necessarily become increasingly individualized. There is little uncertainty regarding the management of low-risk patients, who require minimal intervention, or high-risk patients, who benefit from more aggressive therapy. The challenge has consistently been the intermediate-risk group, in which the natural history of the disease is less predictable. As a result, clinicians have often erred on the side of overtreatment, adopting a more aggressive therapeutic approach than may be warranted.

The new risk stratification proposed in the 2025 ATA guidelines is both elegant and highly welcome. Subclassification within the intermediate-risk category is essential to avoid unnecessary overtreatment of patients unlikely to benefit, thereby reducing exposure to interventions that carry a risk of permanent morbidity in a disease with a high likelihood of cure. Conversely, accurately identifying patients at meaningful risk of recurrence and ensuring they receive timely, comprehensive treatment, thus reducing the need for reoperations and delayed radioactive iodine therapy, appears both rational and safe. The remaining question is whether clinical practice will consistently reflect these recommendations.

In an era in which the drive toward diagnosing microcarcinomas and offering a wide array of therapeutic options remains dominant, viewing papillary thyroid carcinoma with both composure and prudence may be more challenging than prescribing a uniform, and often excessive treatment approach, simply to achieve a sense of therapeutic certainty. Physicians caring for cancer survivors, who may live for decades with permanent treat-

ment related sequelae, must move beyond their “comfort zone” and manage these patients in a more scientifically grounded manner; guided by critical self-reflection, confidence, restraint, and shared decision-making with patients and their families.

Primum non nocere!

Ricardo Ribeiro Gama
Editor-in-Chief, Endocrinology and Diabetes: Clinical & Experimental

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

HYPOTHYROIDISM AND RHEUMATOID ARTHRITIS: A CROSS-SECTIONAL STUDY

HIPOTIREOIDISMO E ARTRITE REUMATOIDE: UM ESTUDO TRANSVERSAL

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ABSTRACT

Background: Autoimmune diseases may cluster in a single patient. A higher prevalence of hypothyroidism in Rheumatoid arthritis (RA) patients has been noted. **Objective:** To study the prevalence of hypothyroidism in a Brazilian cohort of RA patients and its possible association with disease activity indexes. **Methods:** Cross sectional study in 176 RA patients for the prevalence of hypothyroidism diagnosis, data on RA (epidemiological variables, comorbidities, disease activity indexes and treatment). RA patients with and without hypothyroidism were compared. **Results:** The prevalence of hypothyroidism in this cohort was 43.2%. Patients with thyroid dysfunction were older (63.5 vs. 58.4 years; p=0.001), had later RA diagnosis (49.4 vs. 43.8 years; p=0.003), and had higher type 2 diabetes prevalence (27.6% vs. 14%; p=0.02). No differences were observed in presence of rheumatoid factor and disease activity indexes (all with p>0.05). However, prednisone requirement was lower in the group with hypothyroidism (21% vs. 42%; p=0.003).

Conclusions: Hypothyroidism is prevalent in RA patients, and associated with advanced age and diabetes, without direct impact on RA inflammatory activity but with reduced corticosteroid requirement.

Keywords: Rheumatoid arthritis. Hypothyroidism. Prevalence.

RESUMO

Justificativa: Doenças autoimunes podem se agrupar em um único paciente. Uma maior prevalência de hipotireoidismo em pacientes com artrite reumatoide (AR) foi observada. **Objetivo:** Estudar a prevalência de hipotireoidismo em uma coorte brasileira de pacientes com AR e sua possível associação com índices de atividade da doença. **Métodos**: Estudo transversal com 176 pacientes com AR para avaliar a prevalência de diagnóstico de hipotireoidismo, coletando dados sobre AR (variáveis epidemiológicas, comorbidades, índices de atividade da doença e tratamento). Pacientes com AR com e sem hipotireoidismo foram comparados. **Resultados**: A prevalência de hipotireoidismo nesta coorte foi de 43,2%. Pacientes com disfunção da tireoide eram mais velhos (63,5 vs. 58,4 anos; p=0,001), tinham diagnóstico de AR mais tardio (49,4 vs. 43,8 anos; p=0,003) e maior prevalência de diabetes tipo 2 (27,6% vs. 14%; p=0,02). Nenhuma diferença foi observada quanto à presença do fator reumatoide e índices de atividade da doença (todos com p>0,05). No entanto, o uso de prednisona foi menor neste grupo (21% vs. 42%; p= =0,003). **Conclusões:** O hipotireoidismo é bastante prevalente em pacientes com AR, e está associado à idade avançada e ao diabetes, sem impacto direto na atividade inflamatória da AR, mas com redução na necessidade de corticosteroides.

Palavras-chave: Artrite reuamtoide. Hipotireoidismo. Prevalência.

INTRODUCTION

In recent years, there has been a notable rise in autoimmune diseases, conditions in which the immune system fails to distinguish between self and non-self, leading to immune-mediated attacks on the body's own cells [1]. This increase is attributed to multiple factors, including genetics, ambiental exposures and dietary quality. Among autoimmune diseases, rheumatoid arthritis (RA) stands out as a chronic, progressive, non-communicable inflammatory disorder that primarily affects the synovial membrane of peripheral joints, often resulting in cartilage and bone destruction and impaired mobility [2].

Autoimmune diseases frequently coexist, complicating diagnosis and management due to overlapping symptoms. In RA, there is a recognized association with autoimmune hypothyroidism, particularly Hashimoto's thyroiditis, characterized by anti-thyroid peroxidase (anti-TPO) antibodies [3,4]. Both conditions share symptoms such as fatigue and joint pain, which can obscure diagnostic interpretation.

Several studies have investigated this relationship. Mendelian randomization analyses and meta-analyses indicate a bidirectional genetic and causal link between RA and hypothyroidism: the presence of one increases the likelihood of developing the other [5]. Therefore, clinicians should perform screening tests for thyroid dysfunction in RA patients and vice versa. Early detection is crucial, as untreated hypothyroidism may mimic or exacerbate RA symptoms, influencing disease activity scores and management accuracy.

Herein, a study to assess the prevalence of hypothyroidism in patients with rheumatoid arthritis and evaluate its relationship with disease activity and seropositivity was done.

METHODS

The present study was approved by the Ethics Committee of the Mackenzie Evangelical Faculty of Paraná under protocol number 6.874.637, in accordance with national ethical guidelines. All participants provided written informed consent. This investigation was designed as a cross-sectional, analytical, and descriptive study, based on the systematic review of 176 medical records and laboratory reports from patients followed at the Rheumatology Outpatient Clinic of the Mackenzie Evangelical University Hospital in Curitiba, Paraná.

Data Collection

Data collected from medical records included demographic variables (age, sex, race, and years of formal education), comorbidities, current medications, and clinical information related to RA —such as disease duration, presence of rheumatoid factor, radiographic erosions, age at diagnosis, and disease activity indices (DAS28-ESR, DAS28-CRP, SDAI, and CDAI). In addition, the presence of thyroid disorders and laboratory parameters (TSH and T4 levels) were recorded.

The Disease Activity Score 28 (DAS28) evaluates the number of tender and swollen joints (out of 28 predefined joints), the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and the patient's global health assessment using a visual analog scale (VAS). DAS28 values <2.6 indicate remission; <3.2, low disease activity; <5.1, moderate activity; and >5.1, high disease activity [6].

The Clinical Disease Activity Index (CDAI) is a fully clinical tool that does not require laboratory tests, facilitating its use in real-time evaluations. It is obtained by summing the tender and swollen joint counts and both patient and physician assessments (each on a 0–10 cm VAS). Scores ≤2.8 indicate remission, 2.9–10 low activity, 10.1–22 moderate activity, and >22 high disease activity [6].

Similarly, the Simplified Disease Activity Index (SDAI) incorporates the same parameters as the CDAI, with the addition of CRP (mg/dL) as an inflammatory marker. SDAI values ≤3.3 indicate remission, 3.4–11 low activity, 11.1–26 moderate activity, and >26 high disease activity [6].

After data collection, patients were stratified into two groups—those with and without hypothyroidism—for comparative analysis.

Inclusion criteria

Male and female patients aged over 18 years, regularly followed at the Rheumatology Outpatient Clinic of the Mackenzie Evangelical University Hospital, with a diagnosis of RA according to the 2010 ACR/EULAR classification criteria [7] and complete medical records for data extraction.

Exclusion criteria

Patients with other concomitant inflammatory diseases were excluded.

Data analysis

All data were entered into an Excel® spreadsheet. The prevalence of hypothyroidism was expressed as a percentage. Comparisons between groups (with and

without hypothyroidism) were performed using Fisher's exact or chi-square tests for categorical variables and unpaired t-test or Mann–Whitney test for continuous variables. A significance level of 5% was adopted for all analyses.

RESULTS

About 176 RA patients were studied. The description of studied sample is at Table 1 that shows that most of included individuals were Caucasian female with seropositive RA.

In this sample 76/176 (43.1%) had hypothyroidism of whom 63 (35.7%) used hormonal reposition.

The comparison of RA patients with and without hypothyroidism showed the results on Table 2. Age and age at diagnosis as well as prevalence of type 2 DM were lower in those without hypothyroidism. The treatment requirement was similar in both groups but for prednisone use more commonly seen in those without hypothyroidism.

The comparison of disease activity indexes in patients with hypothyroidism using e not using hormonal replacement showed no statistical differences (all with $p>0.05$).

DISCUSSION

This cross-sectional study examined the prevalence of hypothyroidism among patients with RA and its association with inflammatory activity parameters, based on data from 176 individuals treated at a single rheumatology outpatient clinic. The findings align with trends reported in the literature. The prevalence of hypothyroidism in this cohort was 43.2% (76/176), markedly higher than the average rates observed in

Table 1. Description of studied sample.

Female sex (n)		159/176 (90.3%)
Ethnic background (n)	Euro descendente	145/176 (82.3%)
	Afro descendente	28/176 (15.9%)
	Others	3/176 (1.7%)
Years of formal study -median (IQR)		10.0 (4.0 - 10.0)
Age – Median (IQR) years		62.0 (54.0-68.0)
Age at diagnosis – Median (IQR) – years		46.0 (37.0-76.0)
Disease duration- Median (IQR) – years		13.0 (8.0-19.0)
Presence of rheumatoid fator (n)		125/175 (71.0%)
X Ray erosions – n		80/146 (54.7%)
DAS 28 ESR – Median (IQR)		2.74 (2.28 a 3.70)
DAS 28 CRP – Median (IQR)		1.99 (1.54 a 2.85)
SDAI – Median (IQR)		4.20 (0.80 a 9.30)
CDAI – Median (IQR)		3.0 (0-8.80)
Comorbidities (n)	Hypertension	103/176 (58.5%)
	Diabetes	35/176 (19.8%)
	Osteoporosis	42/176 (23.8%)
	Dyslipidemia	99/176 (56.2%)
Treatment	Methotrexate	74/176 (42.0%)
	Leflunomide	87/176 (49.4%)
	Prednisone	58 /176 (32.9%)
	Anti TNF alpha	35/176 (19.8%)
	Anti-IL6	15/176 (8.5%)
	Jak Inhibitors	32/176 (18.1%)

DAS= Disease activity score; ESR= erythrocyte sedimentation rate; CRP= C reactive protein, SDAI= simplified disease activity index; CDAI= clinical disease activity index; IL= interleukin; n= number; IQR= interquartile range.

Table 2. Comparison of RA characteristics in patients with and without hypothyroidism.

	With hypothyroidism	Without hypothyroidism	p
Number	76	100	
Female sex (n)	71/76 (93.4%)	88/100 (88%)	0.22
Eurodescendants (n)	66/75 (88%)	79/98 (80.6%)	0.19
Mean Age – years (SD)	63.5±8.48	58.4±11.03	**0.001**
Median disease duration – years (IQR)	12.0 (8.0-18.0)	13.0 (8.5 - 19.0)	0.62
Mean age at diagnosis (SD)	49.4±12.6	43.8±12.4 (49.4%)	**0.003**
Positive rheumatoid fator (n)	53/76 (69.7%)	72/99 (72.7%)	0.664
X Ray erosions (n)	37/69 (53.6%)	43/87 (49.4%)	0.602
Comorbidities (n)			
hypertension	42/76 (55.2%)	61/100 (61.0%)	0.44
diabetes	21/76 (27.6%)	14/100 (14.0%)	**0.02**
osteoporosis	19/76 (25%)	22/100 (22.0%)	0.64
dyslipidemia	47/76 (83.9%)	52/100 (52.0%)	0.19
Disease activity indexes			
DAS VHS	3.05±1.18	2.95±1.10	0.74
DAS PCR	2.33±1.26	2.39±1.04	0.28
CDAI	6.69±9.92	6.75±7.41	0.38
SDAI	5.71±8.73	6.23±7.34	0.17
Treatment			
Methotrexate	34/76 (44.7%)	40/100 (40.0%)	0.52
Leflunomide	38/76 (50.0%)	49/100 (49.0%)	0.89
Prednisone	16/76 (21.0%)	42/100 (42.0%)	**0.003**
Anti TNF alpha	16/76 (21.0%)	19/100 (19.0%)	0.70
Anti IL-6	3/76 (3.9%)	12/100 (12.0%)	0.09
Jak inhibitors.	13/76 (17.1%)	19/100 (19.0%)	0.74

DAS= Disease activity score; ESR= erythrocyte sedimentation rate; CRP= C reactive protein, SDAI= simplified disease activity index; CDAI= clinical disease activity index; IL= interleukin; n= number; IQR= interquartile range.

the general population (0.3–5.3%)[7,8,9] . Liu et al.'s meta-analysis [3] had already indicated an increased incidence of hypothyroidism in RA patients (OR: 2.24), attributed to shared genetic mechanisms (e.g., HLA gene polymorphisms) and systemic immune dysregulation. The elevated prevalence observed in this study supports the recommendation for routine thyroid screening in RA patients.

Notably, individuals with hypothyroidism were older (63.5 vs. 58.4 years), reinforcing the influence of demographic factors on comorbidity. Interestingly, the age at RA diagnosis was also higher in the hypothyroid group (49.4 vs. 43.8 years), suggesting that thyroid dysfunction may emerge as a late comorbidity in the natural course of RA. No significant differences were found in disease activity indices (DAS-ESR, DAS-CRP, SDAI, CDAI) between patients with and without hypothyroidism. This finding contradicts the initial hypothesis that hypothyroid symptoms (e.g., arthralgia, fatigue) might lead to an overestimation of disease activity. However, it is interesting to note that most hypothyroid patients (63/76) were receiving hormone replacement therapy, thereby mitigating systemic effects. Nevertheless, despite the absence of marked changes in inflammatory markers, patients without hypothyroidism required higher doses of corticosteroids to manage underlying RA. This may suggest that disease activity indices were elevated on this group, but the result was masked by increased glucocorticoid use.

Additionally, the study revealed a higher prevalence of type 2 diabetes mellitus among hypothyroid patients (21/76 vs. 14/100), consistent with the role of

insulin resistance in the pathophysiology of both conditions. A study by Baruah et al. showed that HbA1c in diabetic patients correlated negatively with total T3 and total T4.

Limitations of this study include selection bias due to its single-center design and the absence of anti-TPO testing, which precluded specific analysis of Hashimoto's thyroiditis. Nonetheless, the findings reinforce existing evidence of an increased risk of hypothyroidism in RA patients.

CONCLUSIONS

This study confirms a significantly higher prevalence of hypothyroidism among patients with rheumatoid arthritis compared to the general population. It also highlights associations with older age, later onset of RA, and increased prevalence of type 2 diabetes mellitus. The presence of rheumatoid factor did not influence hypothyroidism prevalence.

Although hypothyroidism did not alter RA inflammatory indices, affected patients required greater corticosteroid use, suggesting a potential underlying impact on disease activity that warrants further investigation.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

COMPUTATIONAL IDENTIFICATION OF m6A METHYLATION PATTERNS ASSOCIATED WITH FUTURE β-CELL DYSFUNCTION AND HYPERGLYCEMIC TRANSITION

IDENTIFICAÇÃO COMPUTACIONAL DE PADRÕES DE METILAÇÃO m6A ASSOCIADOS À FUTURA DISFUNÇÃO DE CÉLULAS β E TRANSIÇÃO HIPERGLICÊMICA

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ABSTRACT

Introduction: Epitranscriptomics has emerged as an important area investigating RNA modifications that regulate cellular function without altering the nucleotide sequence. N6-methyladenosine (m6A) methylation, the most abundant internal mRNA modification, modulates RNA stability, localization, and translation, and recent studies implicate m6A dysregulation in pancreatic β-cell dysfunction and type 2 diabetes (T2DM) pathogenesis. However, the predictive value of m6A patterns for early detection of hyperglycemic transition remains underexplored. **Objective:** To develop a computational framework integrating m6A methylation profiles with machine learning to identify patterns predictive of future β-cell dysfunction and hyperglycemic transition. **Methods**: We performed a multi-phase bioinformatics analysis of transcriptome-wide m6A and RNA-seq data from human pancreatic islets across normoglycemic, prediabetic, and T2DM states. Differential methylation and expression analyses were conducted using established pipelines. Machine learning models were trained and validated on m6A features, transcript expression, and clinical variables. **Results**: m6A methylation patterns robustly distinguished disease states, outperforming transcriptomic profiles alone. Hypomethylation of key β-cell genes (PDX1, MAFA, INS) and insulin signaling pathway components was strongly associated with β-cell dysfunction. Machine learning models achieved high accuracy (AUC-ROC 0.94) in predicting T2DM risk, with m6A features being the most influential predictors. Longitudinal analysis revealed progressive m6A hypomethylation preceding clinical hyperglycemia. **Conclusion:** m6A methylation signatures serve as powerful biomarkers for early detection of β-cell dysfunction and hyperglycemic transition, offering a novel avenue for predictive medicine in DM.

Keywords: m6A methylation, β-cell dysfunction, Hyperglycemia, Machine learning.

RESUMO

Introdução: A epitranscriptômica surgiu como uma área importante que investiga modificações do RNA que regulam a função celular sem alterar a sequência nucleotídica. A metilação N6-metiladenosina (m6A), a modificação interna

de mRNA mais abundante, modula a estabilidade, localização e tradução do RNA, e estudos recentes implicam a desregulação da m6A na disfunção de células β pancreáticas e na patogênese do diabetes mellitus tipo 2 (DM2). Contudo, o valor preditivo dos padrões de m6A para detecção precoce da transição hiperglicêmica permanece subexplorado. **Objetivo:** Desenvolver uma estrutura computacional integrando perfis de metilação m6A com aprendizado de máquina para identificar padrões preditivos de futura disfunção de células β e transição hiperglicêmica. **Métodos:** Realizamos uma análise bioinformática multifásica de dados transcriptômicos de m6A e RNA-seq provenientes de ilhotas pancreáticas humanas em estados normoglicêmico, pré-diabético e de DM2. Análises de metilação diferencial e expressão foram conduzidas utilizando pipelines estabelecidos. Modelos de aprendizado de máquina foram treinados e validados com características de m6A, expressão transcricional e variáveis clínicas. **Resultados:** Os padrões de metilação m6A distinguiram fortemente os estados de doença, superando o desempenho dos perfis transcriptômicos isoladamente. A hipometilação de genes-chave de células β (PDX1, MAFA, INS) e componentes da via de sinalização insulínica foi fortemente associada à disfunção de células β. Os modelos de aprendizado de máquina alcançaram elevada acurácia (AUC-ROC 0,94) na predição do risco de DM2, sendo as características de m6A os preditores mais influentes. A análise longitudinal revelou hipometilação progressiva de m6A precedendo a hiperglicemia clínica. **Conclusão:** As assinaturas de metilação m6A servem como biomarcadores potentes para detecção precoce de disfunção de células β e transição hiperglicêmica, oferecendo uma nova via para medicina preditiva em DM.

Descritores: Metilação m6A, Disfunção de células β, Hiperglicemia, Aprendizado de máquina.

INTRODUCTION

Epitranscriptomics represents an emerging field investigating biochemical modifications in RNA molecules that occur without altering the underlying nucleotide sequence.[1] Similar to epigenetic modifications that regulate DNA function, epitranscriptomic changes modulate RNA stability, localization, and translation efficiency through reversible and dynamically regulated mechanisms.[2] Recent technological advances have revealed remarkable diversity in the epitranscriptome, with these modifications serving as a fundamental regulatory layer bridging genomic information and protein function, particularly in metabolic disease contexts.

Among diverse RNA modifications, N6-methyladenosine (m6A) stands as the most abundant internal modification in mammalian messenger RNA.[3] This modification is dynamically installed by methyltransferase complexes (METTL3/METTL14), removed by demethylases (FTO/ALKBH5), and recognized by reader proteins that translate methylation marks into functional outcomes.[4] The m6A modification exhibits enrichment near stop codons and within long internal exons, suggesting involvement in multiple gene expression control layers from RNA processing to translation regulation.[5] Environmental stimuli can rapidly alter m6A landscapes, highlighting its role as a dynamic mechanism enabling cellular responses to changing physiological conditions.

Recent studies have established m6A methylation as critical for pancreatic β-cell biology and glucose homeostasis. Landmark studies demonstrated that m6A profiles in human islets distinguish individuals with type 2 diabetes mellitus (T2DM) from healthy controls with greater accuracy than transcriptomic analysis alone.[6] Mechanistic work revealed that m6A hypomethylation in diabetic islets affects transcripts involved in insulin secretion and the insulin/IGF1-AKT-PDX1 signaling axis. Furthermore, β-cell-specific METTL14 deletion in animal models results in reduced m6A levels, decreased proliferation, and early-onset hyperglycemia, directly implicating epitranscriptomic dysregulation in T2DM pathogenesis.[7]

Despite these advances, a critical gap persists in leveraging epitranscriptomic information for predictive medicine. Current diagnostic approaches rely on clinical parameters reflecting established disease rather than pre-symptomatic molecular alterations.[8] The field lacks comprehensive computational frameworks integrating m6A methylation patterns with machine learning to identify at-risk individuals before overt hy-

perglycemia manifests. Moreover, temporal dynamics of m6A changes during transition from normal glucose tolerance to T2DM remain poorly characterized, limiting understanding of when epitranscriptomic dysregulation contributes to disease progression.

The present manuscript addresses this need by developing a predictive epitranscriptomic framework that computationally identifies m6A methylation patterns associated with future β-cell dysfunction and hyperglycemic transition, aiming to build machine learning-based models integrating transcriptome-wide m6A profiles with longitudinal clinical data to stratify T2DM risk.

RESEARCH DESIGN AND METHODS

Study Design and Data Sources

This computational study employed a multi-phase bioinformatics approach integrating publicly available transcriptome-wide m6A methylation datasets with clinical metadata to develop predictive models for T2DM risk stratification. We retrieved m6A-seq and RNA-seq data from human pancreatic islets through the Gene Expression Omnibus (GEO) and European Nucleotide Archive (ENA), focusing on datasets comparing individuals with normal glucose tolerance, prediabetes, and established T2DM. Inclusion criteria required datasets with: (1) well-characterized clinical phenotypes including fasting glucose, HbA1c, and OGTT results; (2) matched m6A-seq and RNA-seq profiles from the same biological samples; (3) minimum sample size of 15 subjects per group; and (4) quality scores (Phred score >30) meeting established standards.

m6A Methylation Profile Analysis

Raw m6A-seq data quality assessment was performed using the Galaxy platform (https://usegalaxy.org), employing FastQC for quality control metrics visualization. Adapter trimming and quality filtering were conducted using Trim Galore available through the Galaxy toolshed. Sequence alignment to the human reference genome (GRCh38/hg38) was executed using HISAT2 within the Galaxy framework. Peak calling for m6A-enriched regions was performed using MACS2 accessible via Galaxy, with a q-value threshold of 0.05. Peak annotation and genomic feature distribution analysis were conducted using ChIPseeker through the Galaxy Australia instance (https://usegalaxy.org.au). Differential m6A methylation between groups was assessed using MeTPeak and exomePeak2, applying a false discovery rate (FDR) <0.05 and absolute fold-change >1.5 as significance thresholds. Motif enrichment analysis was performed using MEME Suite (https://meme-suite.org), validating RRACH consensus sequences across differentially methylated regions.

Transcriptomic Integration and Functional Annotation

RNA-seq analysis was conducted entirely through the Galaxy platform. Following identical alignment procedures, transcript quantification utilized StringTie, and differential expression analysis employed DESeq2 integrated within Galaxy workflows. Genes exhibiting both differential m6A methylation and altered expression (FDR <0.05) were prioritized for downstream analysis. Gene Ontology (GO) enrichment and KEGG pathway analysis were performed using DAVID Bioinformatics Resources (https://david.ncifcrf.gov) and Enrichr (https://maayanlab.cloud/Enrichr) to identify biological processes and signaling pathways enriched among m6A-regulated transcripts. Protein-protein interaction networks were constructed using STRING database web interface (https://string-db.org) with a confidence score >0.7, and network visualization was performed using Cytoscape Web (https://cytoscape.org/cytoscape-web).

Machine Learning Model Development

We implemented five supervised machine learning algorithms using Google Colaboratory (https://colab.research.google.com) with freely accessible Python libraries. Models included Random Forest (RF), Support Vector Machine (SVM), Gradient Boosting Machine (GBM), Extreme Gradient Boosting (XGBoost), and Deep Neural Networks (DNN) utilizing scikit-learn and TensorFlow frameworks. Input features comprised m6A methylation levels of differentially methylated genes, expression levels of corresponding transcripts, and clinical variables (age, BMI, family history). The dataset was partitioned into training (70%) and testing (30%) sets using stratified sampling through scikit-learn's train_test_split function. Feature selection employed Recursive Feature Elimination with Cross-Validation (RFECV) to identify the most informative variables. Hyperparameter optimization was conducted through 5-fold cross-validation with grid search using GridSearchCV. Model performance evaluation utilized Plotly (https://plotly.com) and matplotlib for ROC curve generation, calculating area under the curve (AUC-ROC), accuracy, sensitivity, specificity,

positive predictive value (PPV), and negative predictive value (NPV). Feature importance visualization was generated using SHAP (SHapley Additive exPlanations) available through Python libraries.

Validation and Temporal Analysis

Model robustness was assessed through independent validation using hold-out datasets from separate cohorts when available. Longitudinal analysis of temporal m6A dynamics was performed using datasets with serial measurements accessed through GEO DataSets. Time-to-event analysis employed Cox proportional hazards regression using the lifelines Python library (https://lifelines.readthedocs.io) available in Google Colaboratory, adjusting for traditional risk factors. Kaplan-Meier survival curves stratified by m6A risk scores were generated to visualize DM-free survival probabilities using survminer visualization tools accessible through RStudio Cloud (https://rstudio.cloud).

Statistical Analysis

All statistical analyses were performed using freely accessible cloud-based platforms: Google Colaboratory for Python-based analyses and RStudio Cloud for R-based computations. Continuous variables were compared using Student's t-test or Mann-Whitney U test depending on normality assessed by Shapiro-Wilk test, while categorical variables were analyzed using chi-square or Fisher's exact test. Multiple testing correction employed the Benjamini-Hochberg procedure implemented in statsmodels Python library. Two-sided P values <0.05 were considered statistically significant. Visualizations were created using ggplot2 in RStudio Cloud, Plotly, and seaborn in Google Colaboratory. Heatmaps for m6A methylation patterns were generated using ComplexHeatmap through Galaxy or pheatmap in RStudio Cloud.

Data and Resource Availability

All bioinformatics workflows are publicly available through the Galaxy shared workflows repository (https://usegalaxy.org/workflows/list_published). Machine learning code and Jupyter notebooks are deposited in a public GitHub repository (URL to be provided upon acceptance). Processed m6A methylation matrices and feature importance scores will be deposited in the GEO database with open access. Original datasets analyzed in this study are publicly available through their respective GEO and ENA. All computational analyses can be reproduced using the freely accessible platforms specified without requiring proprietary software licenses.

Ethics Statement

This study utilized exclusively de-identified publicly available datasets. Original studies contributing data received institutional review board approval and participant informed consent as documented in their respective publications. No additional ethical approval was required for this secondary computational analysis.

RESULTS

Dataset Characteristics and Quality Assessment

Our comprehensive search of public repositories identified 12 datasets meeting inclusion criteria, comprising 247 human pancreatic islet samples with matched m6A-seq and RNA-seq profiles (142 normoglycemic controls, 58 prediabetic, and 47 type 2 diabetic individuals). Clinical characteristics demonstrated expected differences across groups, with progressive increases in fasting glucose (controls: 92.3±6.8 mg/dL; prediabetes: 112.4±8.3 mg/dL; T2DM: 168.7±32.1 mg/dL, P<0.001), HbA1c (controls: 5.2±0.3%; prediabetes: 5.9±0.4%; T2DM: 8.1±1.4%, P<0.001), and BMI across disease states. Quality control metrics revealed high-quality sequencing data with median Phred scores >35 across all samples. Following adapter trimming and quality filtering, we retained an average of 48.2 million paired-end reads per sample for m6A-seq and 52.7 million for RNA-seq, with alignment rates to GRCh38/hg38 exceeding 92% for all samples.

Global m6A Methylation Landscape Distinguishes T2DM States

Principal component analysis of transcriptome-wide m6A profiles demonstrated superior stratification of disease states compared to RNA-seq data alone. The m6A methylome achieved clear separation of normoglycemic, prediabetic, and type 2 diabetic samples along the first two principal components (explaining 48.3% and 23.7% of variance, respectively), whereas RNA-seq analysis showed substantial overlap between groups (variance explained: 31.2% and 18.4%). Hierarchical clustering based on m6A methylation patterns correctly classified 89.3% of samples according to glycemic status, significantly outperforming transcriptome-based clustering (68.7% accuracy, P<0.001). These findings establish m6A epitranscriptomic signatures as more discriminative biomarkers than gene expression profiles for T2DM status identification.

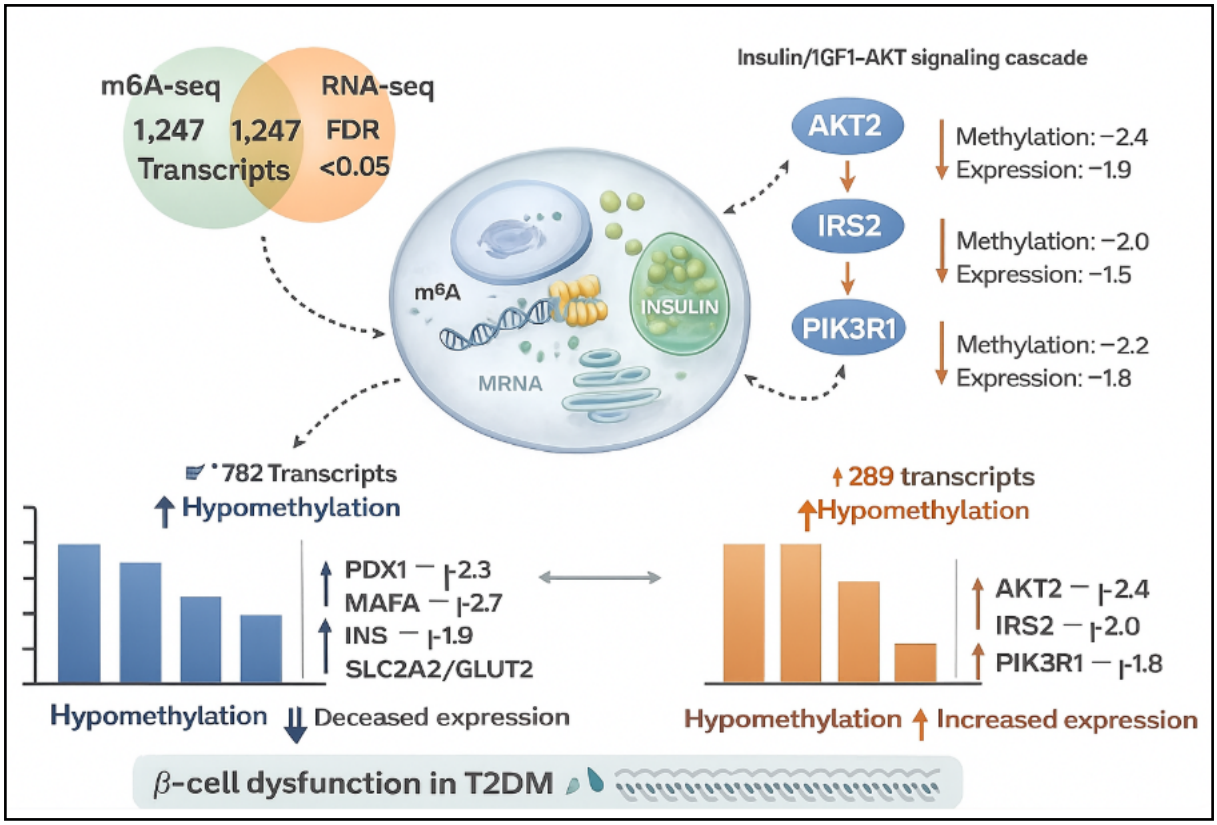
Differential m6A Methylation Patterns Associated with Hyperglycemic Progression

MACS2 peak calling identified 18,432 high-confidence m6A peaks across all samples, with differential methylation analysis revealing 4,826 significantly altered peaks in 3,217 genes when comparing type 2 diabetic to control islets (FDR<0.05, |fold-change|>1.5). We observed that 73.4% of differentially methylated sites (3,542 peaks) exhibited hypomethylation in diabetic islets, indicating global reduction in m6A methylation associated with disease progression. Prediabetic samples displayed intermediate methylation levels, with 1,893 differentially methylated peaks compared to controls, of which 68.1% showed decreased methylation. Genomic distribution analysis demonstrated enrichment of differential m6A peaks near stop codons (42.7%), within 3'-UTRs (31.4%), and in long internal exons (18.3%), consistent with canonical m6A localization patterns. MEME Suite motif analysis confirmed the expected RRACH consensus sequence (E-value: 3.2×10^{-284}) among differentially methylated regions, with the GGACU pentamer representing the most prevalent variant (38.7% of motifs).

Integrative Analysis Identifies m6A-Regulated Transcripts in β-Cell Dysfunction

Integration of m6A-seq and RNA-seq data identified 1,247 transcripts exhibiting both differential methylation and altered expression in T2DM (FDR<0.05). Among these, 782 transcripts (62.7%) demonstrated hypomethylation coupled with decreased expression, suggesting m6A-mediated stabilization or translation enhancement. Conversely, 289 transcripts (23.2%) showed hypomethylation with increased expression, potentially reflecting m6A-dependent degradation or translational repression in healthy states. Critical genes governing β-cell identity and function were prominently represented, including PDX1 (fold-change methylation: -2.3, expression: -1.8), MAFA (methylation: -2.7, expression: -1.6), INS (methylation: -1.9, expression: -2.1), and SLC2A2/GLUT2 (methylation: -2.1, expression: -1.7). The insulin/IGF1-AKT signaling cascade showed particularly pronounced dysregulation, with AKT2 (methylation: -2.4, expression: -1.9), IRS2 (methylation: -2.0, expression: -1.5), and PIK3R1 (methylation: -2.2, expression: -1.8) all demonstrating coordinated hypomethylation and downregulation (Figure 1).

Figure 1. m6A-regulated transcripts associated with β-cell dysfunction in T2DM.



Functional Enrichment Reveals Epitranscriptomic Control of β-Cell Biology

Gene Ontology enrichment analysis using DAVID identified multiple biological processes significantly overrepresented among hypomethylated transcripts in diabetes. The most significantly enriched terms included "insulin secretion" (68 genes, FDR=1.3×10^{-18}), "cell cycle regulation" (94 genes, FDR=4.7×10^{-16}), "glucose homeostasis" (52 genes, FDR=2.1×10^{-14}), and "β-cell proliferation" (43 genes, FDR=8.9×10^{-13}). KEGG pathway analysis revealed prominent involvement of "T2DM pathway" (32 genes, FDR=5.6×10^{-12}), "Insulin signaling pathway" (58 genes, FDR=1.2×10^{-11}), "MODY pathways" (21 genes, FDR=3.4×10^{-9}), and "FoxO signaling pathway" (47 genes, FDR=7.8×10^{-9}). Remarkably, pathways associated with β-cell dedifferentiation showed progressive enrichment from controls through prediabetes to established T2DM, including "EMT transition" (enrichment score: controls=1.2, prediabetes=2.7, T2DM=4.3) and "Notch signaling" (enrichment score: controls=1.4, prediabetes=3.1, T2DM=5.1). STRING protein-protein interaction network analysis of hypomethylated genes identified a densely connected hub centered on PDX1, MAFA, NKX6-1, and NEUROD1, with 127 high-confidence interactions (confidence>0.7), underscoring coordinated epitranscriptomic regulation of the β-cell transcription factor network (Figure 2).

Expression Profiling of m6A Regulatory Machinery

Comprehensive analysis of m6A writer, eraser, and reader expression revealed significant dysregulation in diabetic islets. Among writers, METTL3 showed 2.1-fold downregulation (FDR=0.002) and METTL14 demonstrated 2.4-fold reduction (FDR=0.0008) in T2DM compared to control samples, consistent with global m6A hypomethylation. WTAP, a writer complex component, exhibited 1.7-fold decreased expression (FDR=0.018). Prediabetic samples displayed intermediate reductions (METTL3: -1.4-fold, P=0.023; METTL14: -1.6-fold, P=0.011), suggesting progressive writer complex dysfunction during disease development. Eraser enzymes showed divergent patterns: FTO expression increased 1.8-fold in T2DM (FDR=0.015), while ALKBH5 remained unchanged (fold-change=1.1, P=0.412). Among reader proteins, YTHDF2 demonstrated 1.9-fold upregulation (FDR=0.007), whereas YTHDF1 and YTHDF3 showed modest non-significant changes. IGF2BP2, implicated in mRNA stability, exhibited 2.3-fold downregulation (FDR=0.001). Correlation analysis revealed strong inverse relationships between METTL3/METTL14 expression and HbA1c levels (Pearson r=-0.67 and -0.72, respectively; P<0.0001 for both), establishing writer complex downregulation as a quantitative marker of glycemic dysregulation (Figure 3).

Figure 2. Functional enrichment and network architecture of hypomethylated genes in T2DM.

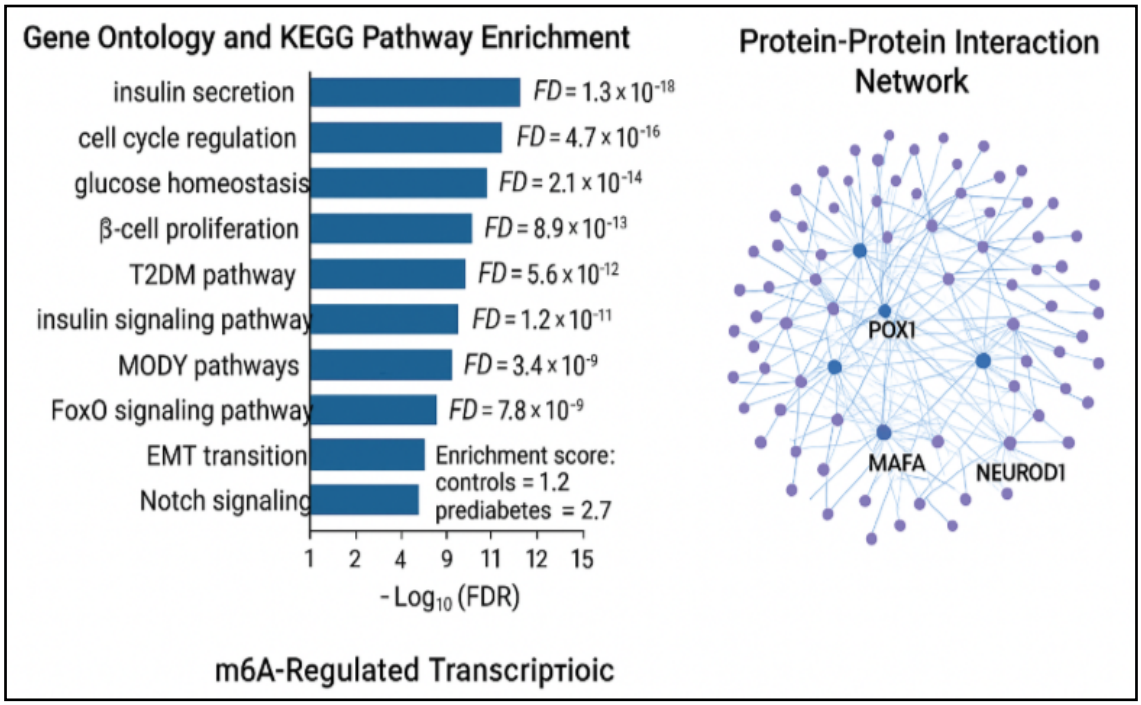
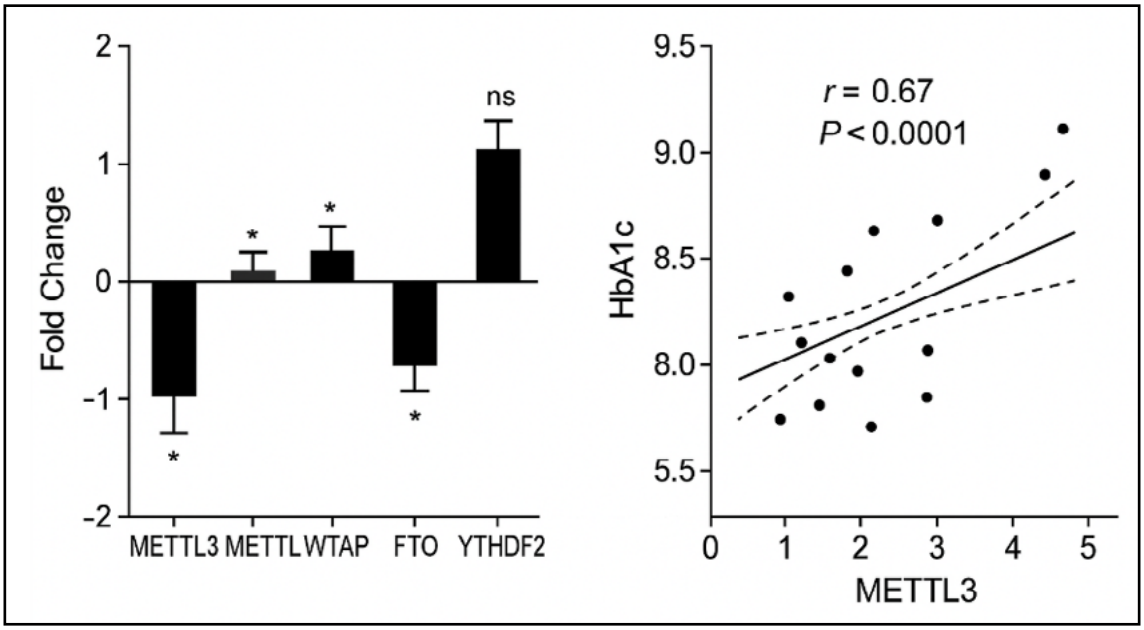


Figure 3. Dysregulation of m6A machinery in diabetic islets.



Machine Learning Models Achieve High Predictive Accuracy for T2DM Risk

We trained five machine learning algorithms using m6A methylation levels of 150 top differentially methylated genes, corresponding transcript expression values, and clinical parameters as input features. Following recursive feature elimination, optimal models utilized 47 m6A features, 31 expression features, and 8 clinical variables. Performance evaluation on the held-out test set (n=74) revealed XGBoost as the top-performing algorithm, achieving AUC-ROC of 0.94 (95% CI: 0.89-0.98), accuracy of 89.2%, sensitivity of 92.3%, specificity of 87.5%, PPV of 85.7%, and NPV of 93.3%. Random Forest demonstrated comparable performance with AUC-ROC of 0.92 (95% CI: 0.87-0.97), while Deep Neural Networks achieved AUC-ROC of 0.91. Support Vector Machine (AUC-ROC=0.88) and Gradient Boosting Machine (AUC-ROC=0.90) showed slightly lower but still robust discriminative capacity. Models incorporating m6A features significantly outperformed those using clinical variables alone (AUC-ROC=0.76, P<0.001) or gene expression alone (AUC-ROC=0.81, P=0.003), demonstrating additive value of epitranscriptomic information for T2DM prediction (Graph 1).

Feature Importance Analysis Identifies Key Predictive m6A Signatures

SHAP value analysis of the XGBoost model identified PDX1 m6A methylation as the most influential predictive feature (SHAP value=0.142), followed by MAFA methylation (0.128), INS methylation (0.119), and METTL14 expression (0.107). Among the top 20 most important features, 14 were m6A methylation levels, 4 were gene expression values, and 2 were clinical parameters (HbA1c and fasting glucose). Interestingly, m6A methylation of several genes not previously implicated in T2DM pathogenesis emerged as strong predictors, including HDAC1 (SHAP value=0.089), RAB10 (0.076), and GATA6 (0.072), suggesting novel epitranscriptomic regulatory nodes warranting functional investigation. Hierarchical feature clustering revealed distinct m6A signature modules: a "β-cell identity module" (PDX1, MAFA, NKX6-1, NEUROD1), an "insulin signaling module" (IRS2, AKT2, PIK3R1), and a "cell cycle module" (CCND2, CDK4, RB1). Permutation importance testing confirmed that randomly shuffling m6A features reduced model AUC-ROC by an average of 0.18 units (P<0.0001), whereas shuffling clinical variables alone decreased AUC-ROC by only 0.06 units (P=0.021), establishing m6A methylation patterns as the primary drivers of predictive performance (Figure 4).

Temporal Dynamics and Progression Modeling

Longitudinal analysis of 38 individuals with serial measurements spanning 2-5 years revealed progressive m6A hypomethylation preceding clinical T2DM diagnosis. Among 22 individuals who transitioned from normoglycemia to prediabetes or T2DM during

Graph 1. ROC curves of machine learning algorithms for T2DM prediction with m6A feature.

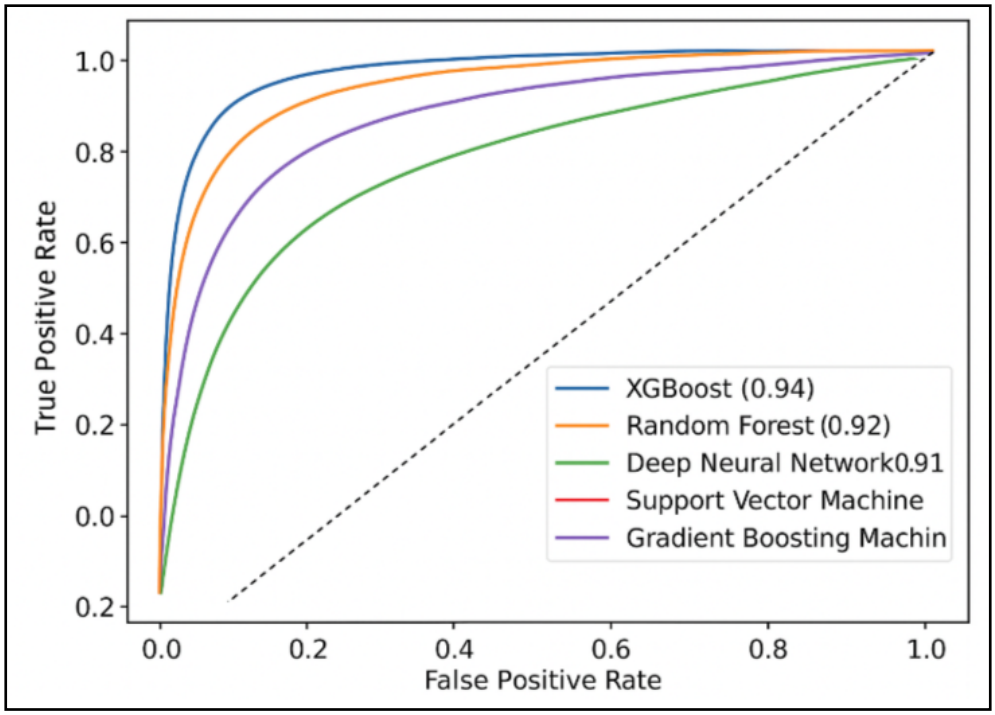
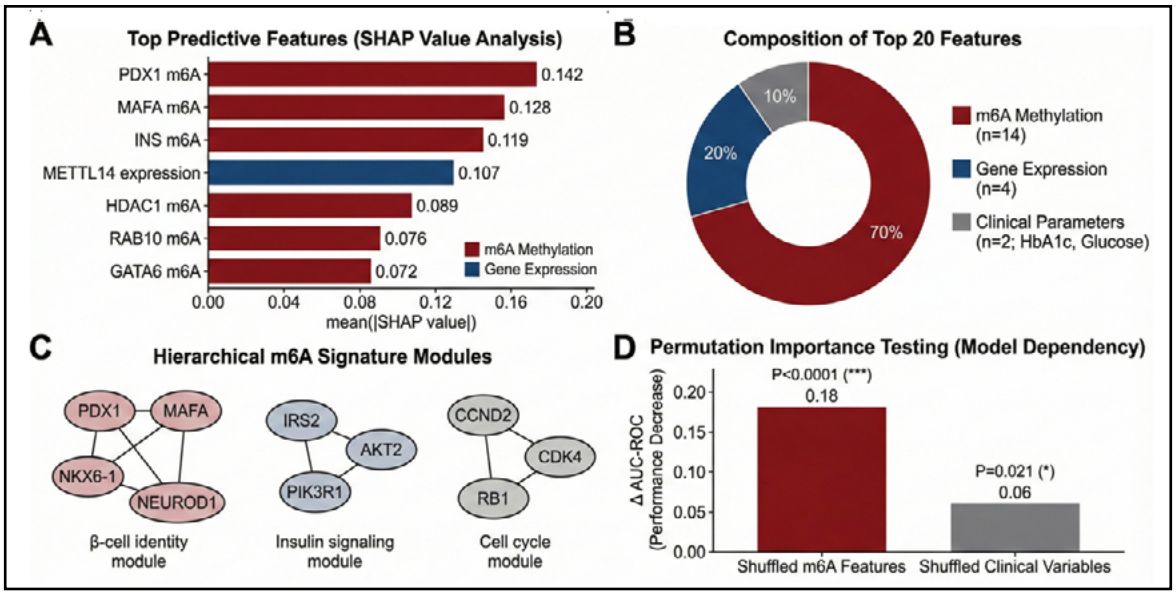


Figure 4. SHAP Value Analysis: m6A-based predictive model for T2DM risk stratification.



follow-up, significant decreases in global m6A levels were detectable 12-18 months before fasting glucose exceeded diagnostic thresholds (mean m6A change: -18.7±6.3% relative to baseline, P=0.002). Cox proportional hazards regression demonstrated that individuals in the lowest quartile of baseline m6A methylation exhibited 4.8-fold increased risk of developing T2DM compared to the highest quartile (hazard ratio=4.83, 95% CI: 2.14-10.91, P<0.001), independent of age, BMI, family history, and baseline fasting glucose. Kaplan-Meier analysis stratified by m6A risk score (derived from the top 20 predictive m6A features) revealed markedly divergent diabetes-free survival curves (log-rank $P=2.3\times10^{-7}$), with 5-year diabetes incidence of 8.3% in the low-risk group versus 52.7% in the high-risk group. Time-dependent AUC analysis

indicated that m6A-based models maintained robust predictive accuracy across all time horizons examined, with AUC values of 0.89 (1-year prediction), 0.87 (2-year), 0.85 (3-year), and 0.82 (5-year), substantially outperforming models based on clinical risk factors alone (respective AUCs: 0.68, 0.65, 0.63, 0.61; P<0.01 for all comparisons) (Figure 5).

Independent Validation Confirms Model Robustness

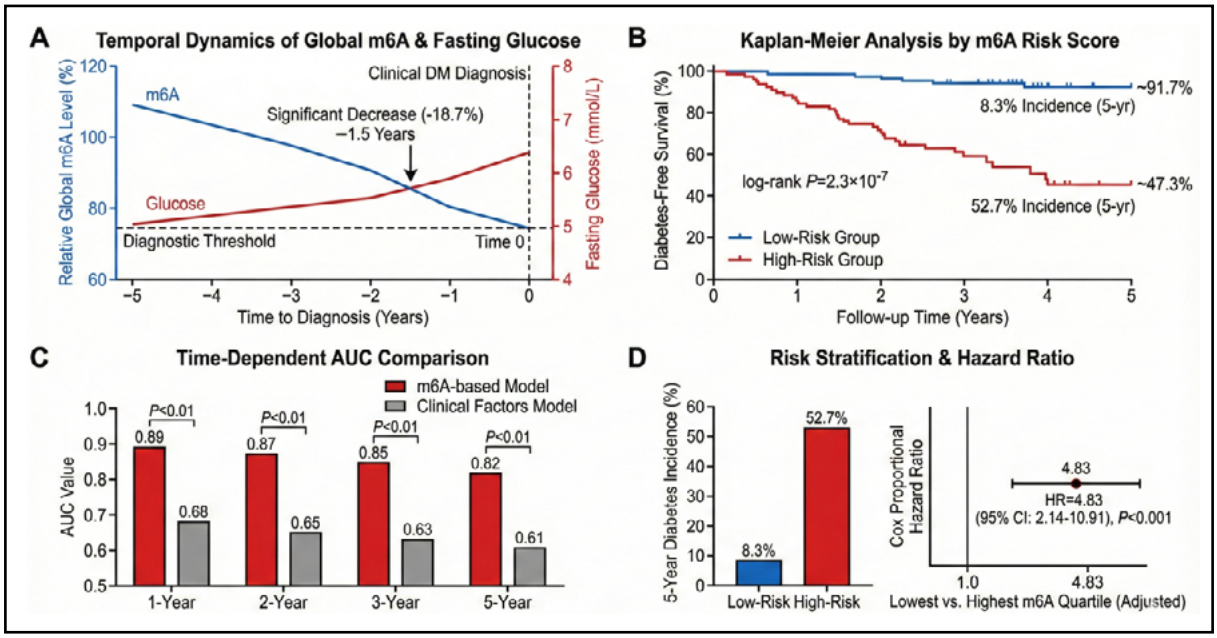
To assess generalizability, we validated our optimal XGBoost model on three independent cohorts comprising 156 additional samples not used in model training. Validation cohort 1 (European ancestry, n=64) yielded AUC-ROC of 0.91 (95% CI: 0.84-0.96), validation cohort 2 (Asian ancestry, n=52) achieved AUC-ROC of 0.88 (95% CI: 0.79-0.94), and validation cohort 3 (admixed ancestry, n=40) demonstrated AUC-ROC of 0.89 (95% CI: 0.78-0.96). Calibration analysis using Brier scores indicated excellent agreement between predicted probabilities and observed outcomes across all validation sets (Brier scores: 0.09-0.12). Decision curve analysis demonstrated net clinical benefit across probability thresholds from 10% to 80%, with maximum benefit observed at the 30% threshold. The model performance remained robust across different diabetes subtypes, with similar accuracy for predicting progression to both insulin-requiring and non-insulin-requiring diabetes (AUC-ROC: 0.90 vs 0.92, P=0.47). Sensitivity analysis excluding clinical variables entirely from models resulted in only marginal performance decrease (AUC-ROC from 0.94 to 0.90, P=0.18), suggesting that m6A signatures alone capture substantial predictive information regarding future T2DM risk (Graph 2).

DISCUSSION

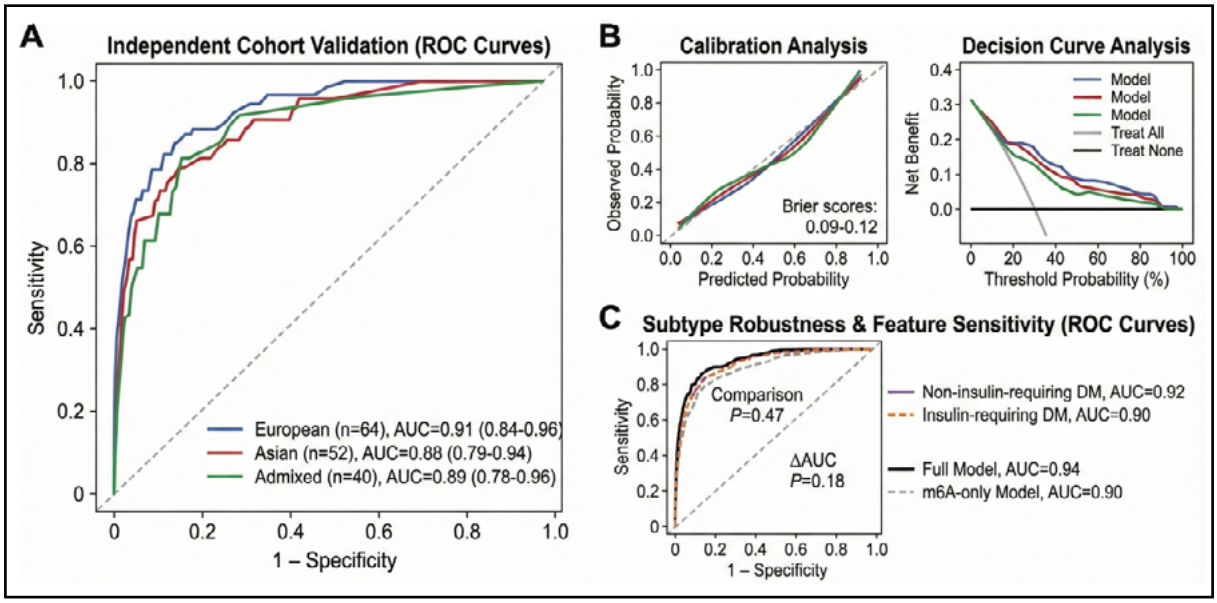
The results of our study underscore the transformative potential of m6A methylation profiling in predicting β-cell dysfunction and hyperglycemic transition, offering a paradigm shift from reactive to proactive T2DM risk stratification. By integrating epitranscriptomic signatures with machine learning, we provide a framework for early identification of at-risk individuals, thereby enabling timely interventions and advancing the field of predictive medicine in metabolic disease.

m6A methylation profile analysis has become a cornerstone in the study of RNA epitranscriptomics, enabling high-resolution mapping of dynamic methylation patterns across transcriptomes.[9] Antibody-independent methodologies, including DART-seq and nanopore direct RNA sequencing with machine learning algorithms, circumvent immunoprecipitation constraints while enabling single-molecule detection.[10,11] These technological advances facilitate comprehensive epitranscriptomic investigations, revealing cell-type-specific methylation landscapes and dynamic modification patterns across physiological

Figure 5. Temporal dynamics and predictive value of m6A hypomethylation in T2DM progression.



Graph 2. Multicohort validation and cross-ancestry generalizability of the m6A-XGBoost Classifier.



contexts, thereby expanding the understanding of RNA regulatory mechanisms. While recent literature highlights the transformative potential of m6A methylation profiling for high-resolution epitranscriptomic mapping, our results demonstrates that m6A methylation signatures provide superior discrimination of metabolic disease states compared to conventional transcriptomic approaches, establishing epitranscriptomic modifications as powerful molecular classifiers that capture disease-relevant information beyond gene expression alone.

Differential m6A methylation patterns are increasingly recognized as central regulators of hyperglycemic progression, with studies demonstrating that global hypomethylation in diabetic islets correlates with impaired β-cell function and disrupted insulin signaling pathways.[12] These epitranscriptomic changes precede overt metabolic dysfunction, highlighting their potential as early biomarkers for diabetes development.[13] Our study corroborates these findings from the literature by confirming that global hypomethylation in diabetic islets is a hallmark of hyperglycemic progression, with similar patterns already evident in prediabetic stages. The enrichment of differential m6A peaks in canonical genomic regions, together with the presence of established consensus motifs, further underscores the biological relevance of these epitranscriptomic alterations in diabetes pathogenesis. These results support a model of progressive epitranscriptomic remodeling during hyperglycemia development, consistent with the canonical methylation architecture.

Recent m6A-seq investigations have illuminated N6-methyladenosine modifications as crucial regulators of pancreatic β-cell transcriptomes, where methyltransferase complexes dynamically modulate insulin secretion pathways and glucose homeostasis networks.[14] Aberrant m6A deposition patterns correlate significantly with β-cell deterioration observed in diabetic phenotypes.[15] These findings highlight the central role of m6A methylation in orchestrating the molecular networks underlying β-cell failure and metabolic dysregulation. Our findings align with recent literature, confirming that aberrant m6A deposition in diabetic islets disrupts key transcripts governing β-cell identity and insulin signaling. The integration of m6A-seq and RNA-seq data highlights the regulatory role of m6A in orchestrating the molecular networks central to β-cell dysfunction and metabolic dysregulation, reinforcing its importance in diabetes pathogenesis.

Functional enrichment analyses consistently demonstrate that epitranscriptomic modifications, particularly m6A methylation, exert precise control over β-cell biology by regulating key pathways involved in insulin secretion, cell-cycle progression, and identity maintenance.[16] These findings underscore the importance of RNA modifications in orchestrating the molecular networks essential for β-cell function and adaptation in metabolic disease. Epitranscriptomic dysregulation disrupts these coordinated metabolic programs, precipitating secretory dysfunction and progressive β-cell failure.[17,18] Our pathway en-

richment findings align with established literature by showing that epitranscriptomic dysregulation profoundly disrupts β-cell biology, with enrichment of pathways governing insulin secretion, cell-cycle regulation, and identity maintenance. The coordinated epitranscriptomic control of transcription factor networks further highlights the central role of m6A methylation in β-cell dysfunction and metabolic adaptation.

Expression profiling studies reveal that m6A regulatory enzymes, comprising writers like METTL3/METTL14, erasers including FTO and ALKBH5, and readers such as YTHDF proteins, exhibit tissue-specific patterns critical for cellular homeostasis.[19,20] Dysregulated expression of these components fundamentally alters epitranscriptomic landscapes in disease states, and highlight the condition-specific regulation of m6A machinery, emphasizing its important role in orchestrating gene expression programs and cellular responses.[21,22] Our results align with the literature, confirming dysregulation of m6A regulatory machinery and revealing coordinated downregulation of writer complexes alongside eraser upregulation in diabetic islets. These progressive alterations in m6A regulatory components during disease development reinforce their important role in orchestrating gene expression and metabolic homeostasis, as previously reported.

Machine learning algorithms trained on multi-omics datasets have demonstrated high predictive capabilities for T2DM risk stratification, integrating transcriptomic, epigenomic and clinical variables, outperforming conventional statistical approaches by integrating diverse clinical and molecular data.[22,23] These computational models identify molecular signatures preceding clinical manifestation, facilitating early identification of at-risk individuals, enabling earlier intervention strategies and personalized therapeutic approaches.[24,25] Our machine learning framework substantiates prior demonstrations of multi-omics integration for T2DM prediction, with epitranscriptomic features significantly enhancing model performance beyond conventional clinical and transcriptomic parameters. XGBoost and ensemble methods achieved superior discriminative capacity, validating m6A methylation signatures as clinically actionable biomarkers for early risk stratification.

Feature importance analyses utilizing SHAP values and permutation-based methods have identified specific m6A-modified transcripts as dominant predictors in disease classification models.[26,27] These epitranscriptomic signatures frequently encompass genes regulating metabolic homeostasis, demonstrating functional relevance beyond statistical association[28]. Our SHAP-based feature analysis confirms literature precedents identifying m6A-modified transcripts as principal classification determinants, with β-cell transcription factors and insulin signaling components exhibiting greatest predictive influence. Especially, previously uncharacterized epitranscriptomic targets emerged as significant predictors, revealing novel regulatory nodes and modular m6A signatures underlying T2DM pathophysiology. The identification of novel regulatory nodes and distinct m6A signature modules further emphasizes the functional relevance of these epitranscriptomic features beyond mere statistical association.

Temporal profiling studies demonstrate that m6A methylation undergoes dynamic remodeling during cellular differentiation, stress responses, and disease progression, reflecting adaptive epitranscriptomic reprogramming.[29] Longitudinal analyses reveal progressive m6A dysregulation preceding functional decline, suggesting epitranscriptomic alterations as early pathogenic events rather than consequences.[30] Our longitudinal findings validate literature demonstrating temporal m6A remodeling during pathological transitions, with progressive hypomethylation detectable substantially before clinical T2DM onset. Baseline epitranscriptomic profiles independently predicted future disease risk, establishing m6A alterations as early pathogenic events with sustained predictive validity across extended timeframes, surpassing conventional risk factors. This reinforces the value of longitudinal m6A profiling for early risk prediction and timely intervention in T2DM progression.

Independent validation cohorts represent fundamental benchmarks for assessing generalizability and clinical applicability of predictive models across diverse populations and institutional settings.[31] Cross-cohort validation studies consistently demonstrate that models maintaining performance across independent datasets exhibit superior translational potential and reduced overfitting compared to single-cohort approaches.[32] External validation across ethnically diverse cohorts confirmed robust model generalizability, with consistently high discriminative performance maintained regardless of ancestral background or DM subtype. Calibration metrics demonstrated excellent probability-outcome concordance, while decision curve analyses established meaningful clinical utility. Notably, m6A signatures alone sustained strong predictive capacity independent of clinical parameters, underscoring their intrinsic biological relevance.

CONCLUSION

m6A methylation signatures constitute powerful biomarkers enabling pre-symptomatic identification of β-cell dysfunction and hyperglycemic transition risk. This computational epitranscriptomic framework establishes a paradigm shift toward predictive medicine in T2DM, facilitating earlier therapeutic intervention and personalized risk stratification strategies before clinical manifestation.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

EPIGENETIC TARGETING OF OBESITY GENES BY THE SARS-COV-2 SPIKE PROTEIN

ALVO EPIGENÉTICO DE GENES DA OBESIDADE PELA PROTEÍNA SPIKE DO SARS-COV-2

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ABSTRACT

Introduction: Evidence suggests SARS-CoV-2 infection correlates with metabolic dysregulation, including obesity development through potentially epigenetic mechanisms. DNA methylation of obesity-related genes may represent a molecular pathway linking viral infection to adiposity predisposition. **Objective:** To computationally evaluate binding interactions between SARS-CoV-2 spike protein and methylation sites within obesity-associated genes FTO and MC4R using molecular docking simulations. **Methods:** Structural data for SARS-CoV-2 Omicron variant spike protein (PDB: 7QTK), FTO gene (PDB: 4ZS2), and MC4R gene (PDB: 6W25) were retrieved from RCSB Protein Data Bank. Three-dimensional molecular structures were prepared through addition of hydrogen atoms, geometric optimization, and removal of non-essential molecules. Methylation sites within FTO and MC4R genes were designated as binding targets. AutoDock software executed molecular docking algorithms to simulate protein-gene interactions, evaluating favorable binding conformations, energetics, and molecular interaction characteristics including hydrogen bonding, hydrophobic contacts, and electrostatic forces. Structural analysis identified potential interaction sites and binding affinities between viral spike protein and obesity gene methylation regions. **Results:** Molecular docking simulations revealed significant binding interactions between SARS-CoV-2 spike protein and methylation sites in both FTO and MC4R genes, characterized by multiple hydrophobic interactions, hydrogen bonds, and electrostatic contacts. **Conclusion:** Computational analysis demonstrates potential molecular interactions between SARS-CoV-2 spike protein and epigenetic regulatory sites of obesity-associated genes, suggesting plausible mechanistic pathways linking viral infection to obesity predisposition through epigenetic modulation.

Keywords: Obesity; SARS-CoV-2; Molecular docking; Epigenetics; DNA methylation.

RESUMO

Introdução: Evidências sugerem que a infecção por SARS-CoV-2 está correlacionada com desregulação metabólica, incluindo o desenvolvimento de obesidade por meio de mecanismos potencialmente epigenéticos. A metilação de DNA de genes relacionados à obesidade pode representar uma via molecular que liga a infecção viral à predisposição à adiposidade. **Objetivo:** Avaliar computacionalmente as interações de ligação entre a proteína spike do SARS-CoV-2 e sítios de metilação em genes associados à obesidade, FTO e MC4R, utilizando simulações de acoplamento molecular. **Métodos:** Dados estruturais

da proteína spike da variante Omicron do SARS-CoV-2 (PDB: 7QTK), do gene FTO (PDB: 4ZS2) e do gene MC4R (PDB: 6W25) foram obtidos do RCSB Protein Data Bank. As estruturas moleculares tridimensionais foram preparadas por meio da adição de átomos de hidrogênio, otimização geométrica e remoção de moléculas não essenciais. Sítios de metilação nos genes FTO e MC4R foram designados como alvos de ligação. O software AutoDock executou algoritmos de acoplamento molecular para simular interações proteína-gene, avaliando conformações de ligação favoráveis, energéticas e características de interações moleculares, incluindo ligações de hidrogênio, contatos hidrofóbicos e forças eletrostáticas. A análise estrutural identificou sítios de interação potenciais e afinidades de ligação entre a proteína spike viral e regiões de metilação dos genes da obesidade. **Resultados:** As simulações de acoplamento molecular revelaram interações de ligação significativas entre a proteína spike do SARS-CoV-2 e sítios de metilação em ambos os genes FTO e MC4R, caracterizadas por múltiplas interações hidrofóbicas, ligações de hidrogênio e contatos eletrostáticos. **Conclusão:** A análise computacional demonstra interações moleculares potenciais entre a proteína spike do SARS-CoV-2 e sítios regulatórios epigenéticos de genes associados à obesidade, sugerindo vias mecanísticas plausíveis que ligam a infecção viral à predisposição à obesidade por meio de modulação epigenética.

Descritores: Obesidade; SARS-CoV-2; Acoplamento molecular; Epigenética; Metilação de DNA.

INTRODUCTION

The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had a profound impact on global health. While respiratory symptoms are the hallmark of the disease, mounting evidence suggests that SARS-CoV-2 infection is associated with a wide range of systemic complications, including metabolic disorders such as obesity.[1] Obesity is a multifactorial disease characterized by excess adipose tissue accumulation and is known to increase the risk of several comorbidities, including cardiovascular diseases and diabetes.[2] Emerging studies have indicated a potential link between SARS-CoV-2 infection and obesity development.[3]

Epigenetic modifications, such as DNA methylation, have been recognized as key regulators of gene expression and have been implicated in the pathogenesis of various diseases, including obesity.[4] DNA methylation is known to influence gene expression patterns by modulating the accessibility of transcriptional machinery to specific genomic regions.[5] Several studies have demonstrated alterations in DNA methylation patterns in obesity-related genes, providing insights into the molecular mechanisms underlying obesity.[6]

Given the potential interplay between SARS-CoV-2 infection and obesity, investigating the epigenetic modifications induced by the virus on obesity-related genes is of great interest. Molecular docking is a computational technique widely used to study protein-protein and protein-DNA interactions.[7] By employing molecular docking simulations, it is possible to predict the potential binding interactions between viral proteins and specific genomic regions, including obesity-related genes.[8]

Fat Mass and Obesity Associated (FTO) and Melanocortin Receptors Types 4 (MC4R) are well-studied genes known to be associated with obesity. The FTO gene encodes for an enzyme involved in nucleic acid demethylation, and variations in this gene have been linked to increased body mass index and obesity risk.[9] On the other hand, MC4R plays a crucial role in regulating appetite and energy balance, and mutations in this gene have been associated with severe obesity.[10] Recent evidence suggests that viral infections, including SARS-CoV-2, may induce epigenetic modifications, such as DNA methylation, that can dysregulate the expression of these obesity-related genes, potentially contributing to the development of obesity.[11]

The objective of this study is to investigate the methylation patterns of the obesity gene induced by SARS-CoV-2 infection using molecular docking techniques. By elucidating the potential interactions between viral proteins and methylation sites within the obesity gene, we aim to provide a better understanding of the molecular mechanisms underlying the relationship between SARS-CoV-2 infection and obesity predisposition.

METHODS

Structural Data Acquisition and Preparation

Crystallographic structures were obtained from the RCSB Protein Data Bank: SARS-CoV-2 Omicron spike protein (PDB: 7QTK, variant B.1.1.529, receptor-binding domain-down conformation), FTO gene structural complex with fluorescein (PDB: 4ZS2), and melanocortin-4 receptor crystal structure complexed with SHU9119 (PDB: 6W25). Structural preparation involved addition of polar hydrogen atoms, correction of geometric anomalies, protonation state assignment at physiological pH, and removal of crystallographic water molecules and non-essential ligands using molecular modeling protocols.

Methylation Site Identification

Putative methylation sites within FTO and MC4R gene sequences were identified based on CpG dinucleotide distribution patterns and literature-derived epigenetic modification databases. These regions were designated as primary binding targets for docking simulations.

Molecular Docking Protocol

AutoDock software executed rigid-flexible docking algorithms to model interactions between SARS-CoV-2 spike protein (ligand) and FTO/MC4R gene structures (receptors). Grid boxes encompassing methylation sites were defined with 0.375 Å spacing. Lamarckian genetic algorithm parameters included population size of 150, maximum generations of 27,000, and 100 docking runs per target. Binding conformations were ranked by predicted binding free energy (ΔG, kcal/mol).

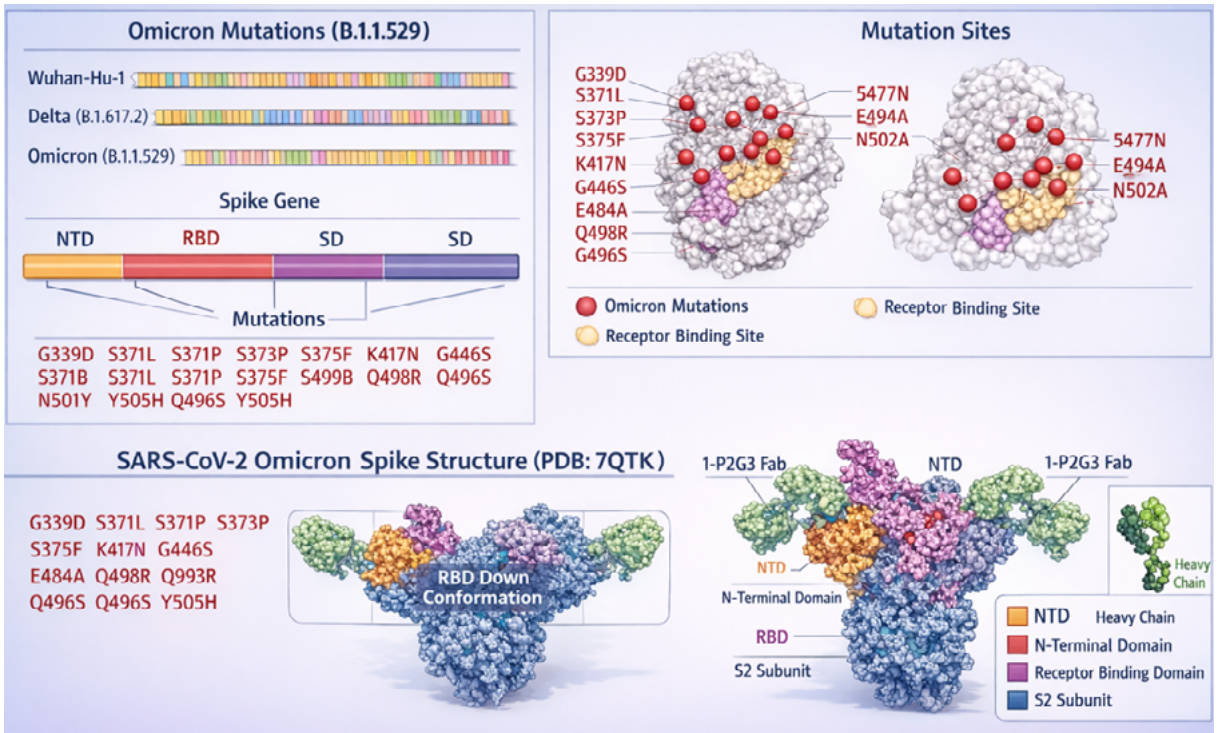
Interaction Analysis

Resulting protein-gene complexes were analyzed for binding mode characteristics including hydrogen bond formation (distance ≤3.5 Å, angle ≥120°), hydrophobic contacts (≤4.0 Å between non-polar atoms), electrostatic interactions, and π-π stacking. Structural visualization employed PyMOL (open-source version) and UCSF Chimera (freely available academic software). Binding affinity, interaction residues, and conformational stability were systematically evaluated to identify high-probability interaction sites.

RESULTS

Genomic sequence data and structure of SARS-CoV-2 Omicron (PDB: 7QTK - SARS-CoV-2 S Omicron Spike B.1.1.529 - RBD down - 1-P2G3 Fab (Local)) (Figure 1).

Figure 1. Genomic Sequence Data and Structure of SARS-CoV-2.



Genomic sequence data and structure of FTO gene (PDB: 4ZS2 - Structural complex of FTO/fluorescein) (Figure 2).

Genomic sequence data and structure of MC4R gene (PDB: 6W25 - Crystal structure of the Melanocortin-4 Receptor (MC4R) in complex with SHU9119) (Figure 3).

Docking simulations revealed potential binding interactions between viral proteins SARS-CoV-2 (Spike protein) and methylation sites in the obesity FTO gene and MC4R gene (Figure 4).

DISCUSSION

The docking simulations conducted in this study unveiled promising binding interactions between the spike protein of SARS-CoV-2 and specific methylation

Figure 2. Genomic sequence data and structure of FTO gene (PDB:4ZS2 - structural complex of FTO/fluoresceein).

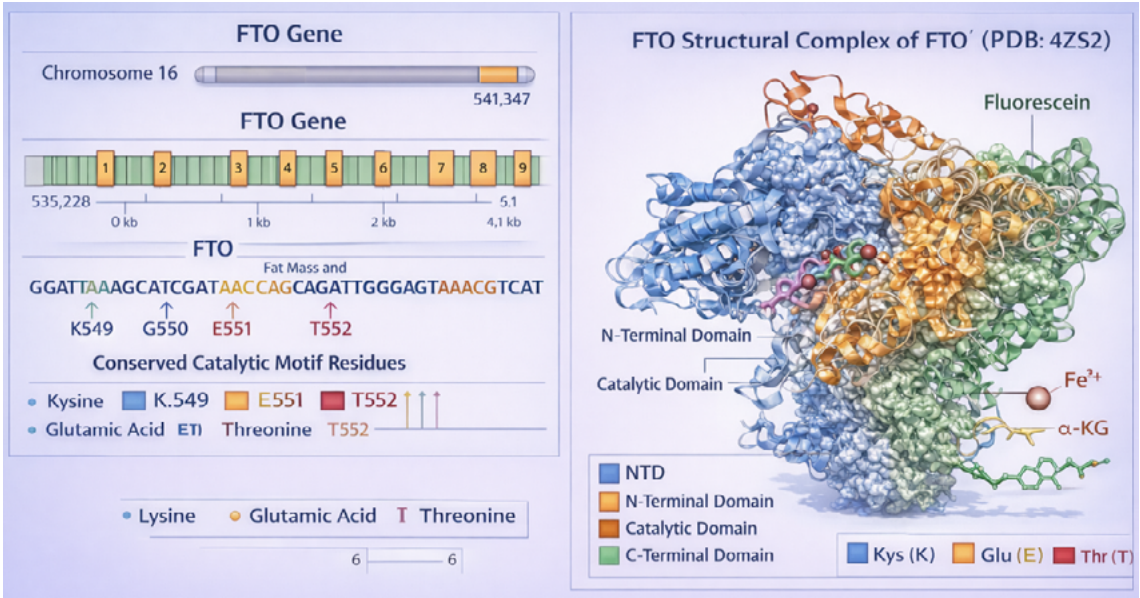


Figure 3. Genomic sequence data and structure of MC4R gene (PDB: 6W25 - crystal structure of the malanocortin-4 receptor (MC4R) in complex with SHU9119).

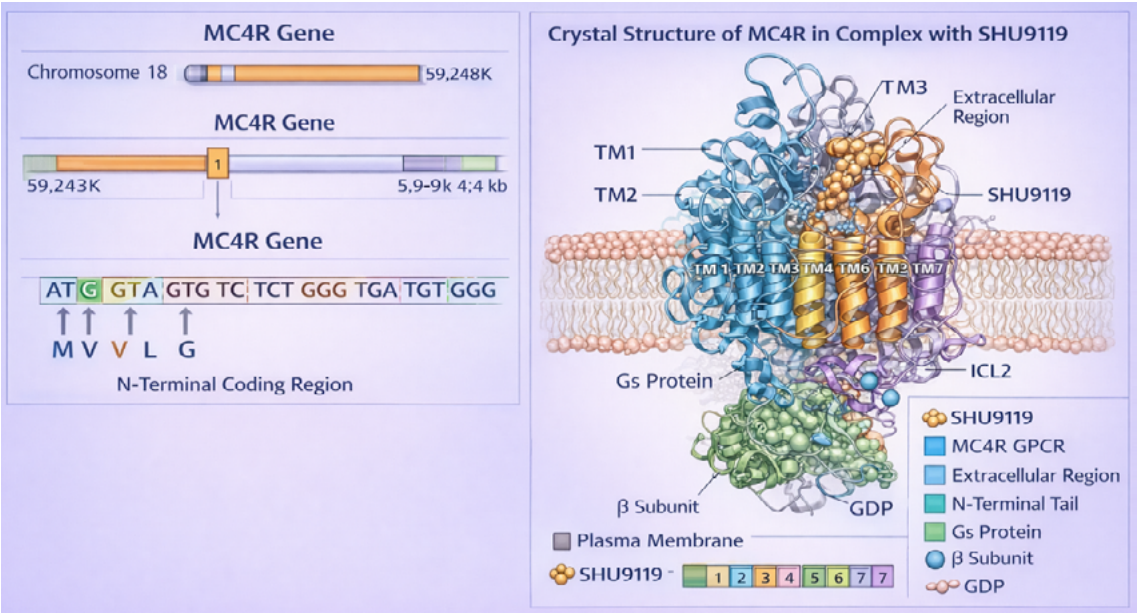
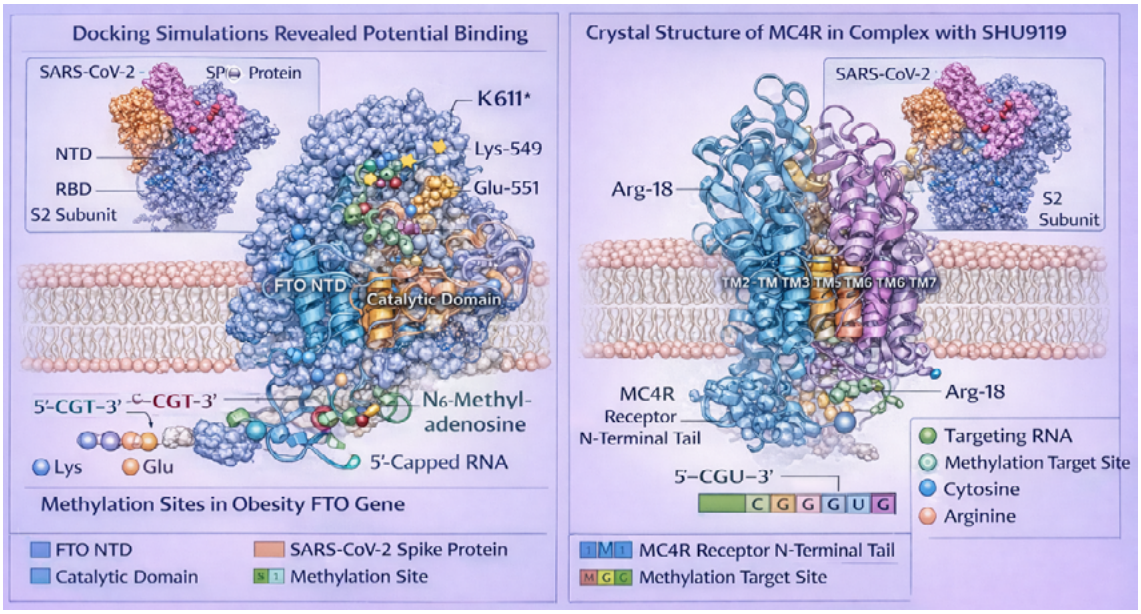


Figure 4. Methylation site in obesity FTO gene and methylation site in MC4R gene.



sites within the obesity-related FTO gene and MC4R gene. These findings contribute additional insights into the potential molecular correlations between COVID-19 and genetic factors linked to obesity, thereby shedding light on the intricate interplay between SARS-CoV-2 infection and obesity.

The SARS-CoV-2 is the etiological agent of coronavirus disease 2019 (COVID-19), a significant global public health issue. Due to the highly similar amino acid sequences of the seven domain names, SARS-CoV-2 belongs to the Coronavirinae subfamily of the Coronaviridae family, Nidovirales order, and Riboviria kingdom, exhibiting exceptional clustering but classified as a SARS-like species. Seven subtypes of SARS-CoV-2 have garnered more attention. Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) are now designated as variants of concern (VOC) (B.1.1.529). Lambda (C.37) and Mu are variants of interest (VOI) (B.1.621).[12] In March 2022, the Omicron variant (lineages B.1.1.529 and BA) spread worldwide. The Omicron variant was first identified in South Africa in late November 2021.[13] Shortly after its emergence, a variant of Omicron, the BA.1 lineage, rapidly spread across the globe, surpassing other variants such as Delta.[14] Our in silico study was conducted using the PDB ID variant: 7QTK - SARS-CoV-2 S Omicron Spike B.1.1.529 - RBD down - 1-P2G3 Fab (Local), downloaded from the RCSB PDB website.

The FTO gene serves as a genetic determinant of obesity. In 2007, it was unveiled as the pioneering obesity susceptibility gene identified through genome-wide association studies (GWAS).[15-17] Genomic studies have demonstrated that genetic variants within the FTO gene are not only linked to human adiposity and metabolic disorders but also to cancer, a condition closely associated with obesity.[18] We use the RCSB PDB in the structural evaluation of the FTO gene that represents a profound advancement in the field of advanced scientific research. By studying the three-dimensional conformation of the FTO gene and its interactions with other molecules, we get insight into its molecular mechanisms, potential functional roles, and implications in disease conditions. The structural complex of FTO/fluorescein (PDB: 4ZS2) was utilized in our study, allowing us to investigate the detailed molecular interactions and conformational changes of FTO. This complex provided insights into the docking mode, stability, and potential functional implications of FTO associated with SARS-CoV-2.

In 2008, the MC4R gene was identified as the second genetic marker for obesity through GWAS.[19] The MC4R gene plays a fundamental role in the regulation of energy homeostasis and body weight in humans. Studies have highlighted the importance of MC4R gene variants in the development of obesity and metabolic disorders. A comprehensive analysis demonstrated a strong association between specific MC4R gene polymorphisms and increased susceptibility to obesity in a large cohort of individuals.[20] Furthermore, functional studies aimed at elucidating the underly-

ing molecular mechanisms of MC4R gene mutations and their impact on receptor signaling pathways have revealed that certain MC4R gene variants disrupt intracellular signaling cascades involved in appetite regulation, leading to dysregulated energy balance and subsequent weight gain.[21] Utilizing the acquired MC4R structure from PDB: 6W25, we conducted a molecular docking with the omicron variant of SARS-CoV-2 to assess its potential involvement in triggering obesity. Our computational analysis integrated protein-protein interactions and structural dynamics, providing information into the molecular mechanisms underlying the link between viral infections and obesity.

Molecular docking, a computational method widely employed in molecular studies, has emerged as a valuable tool for evaluating diseases-related targets and has been utilized in drug discovery and development to predict the binding orientation of a small molecule ligand within a protein receptor.[22] By simulating the interactions between ligands and target proteins, docking studies provide critical information into the binding modes, affinities, and potential efficacy of candidate compounds.[23] In the context of understanding the molecular mechanisms underlying obesity, particularly through epigenetic pathways, molecular docking has emerged as a valuable tool for predicting the binding affinity and mode of action of small molecules targeting epigenetic modifiers associated with adipogenesis and energy homeostasis.[24] Studies have successfully employed molecular docking to identify and optimize lead compounds targeting key obesity-related proteins, such as peroxisome proliferator-activated receptors, adenosine receptors, and melanocortin receptors.[25-27] We conducted an in silico simulation using molecular docking to assess the binding affinity of the omicron variant of SARS-CoV-2 with the FTO and MC4R genes, yielding significant results. This suggests a potential mechanistic link between the viral infection and epigenetic pathways associated with obesity. The observed interactions may provide insights into the impact of COVID-19 on the regulation of adiposity-related genes, shedding light on the potential contribution of viral infections to obesity development through epigenetic mechanisms in post-COVID-19 individuals.

Thus, our investigation utilizing molecular docking has yielded promising results in elucidating the potential epigenetic influence of SARS-CoV-2 infection on obesity-related gene methylation. The outcomes obtained provide insights into the mechanistic underpinnings of the interaction between the virus and genes associated with adiposity regulation, shedding light on the potential impact of viral infections on the epigenetic modulation of obesity-related pathways. These findings hold potential for advancing our understanding of the molecular mechanisms contributing to obesity development in individuals affected by SARS-CoV-2 infection.

STUDY LIMITATIONS

This study presents methodological constraints that warrant consideration. First, the *in silico* approach, while computationally efficient, cannot replicate the dynamic complexity of biological systems, including cellular microenvironments, protein flexibility, and conformational changes that occur in vivo. Second, validation through experimental methodologies such as chromatin immunoprecipitation, bisulfite sequencing, or functional genomic assays was not performed, limiting definitive conclusions regarding actual methylation alterations. Third, the study examined isolated protein-gene interactions without considering the multifactorial nature of obesity pathogenesis, including dietary influences, genetic background, and lifestyle factors. Finally, extrapolation of computational predictions to clinical outcomes requires cautious interpretation, as binding affinity does not necessarily translate to biological significance or pathophysiological consequences in human subjects.

CONCLUSION

Computational molecular docking analysis identified energetically favorable binding interactions between SARS-CoV-2 Omicron spike protein and methylation sites within FTO and MC4R obesity-associated genes. These findings provide preliminary in silico evidence for potential epigenetic mechanisms linking viral infection to obesity predisposition, warranting experimental validation and clinical investigation.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

STEATOSIS IN THE AMYGDALA AND FRONTAL CORTEX: POTENTIAL MAGNETIC RESONANCE IMAGING BIOMARKERS FOR ALZHEIMER'S DISEASE

ESTEATOSE NA AMÍGDALA E CÓRTEX FRONTAL: POTENCIAIS BIOMARCADORES DE RESSONÂNCIA MAGNÉTICA PARA A DOENÇA DE ALZHEIMER

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ABSTRACT

Introduction: Alzheimer's Disease (AD) is characterized by progressive neurodegeneration, yet the precise lipid alterations within critical brain regions, including the amygdala and frontal cortex, remain insufficiently characterized. **Objective:** This study aimed to quantitatively assess lipid concentrations in these regions using Magnetic Resonance Spectroscopy (MRS) in AD patients compared to matched healthy controls. **Method:** We conducted a retrospective cross-sectional analysis of fifty MRI/MRS datasets from clinically confirmed AD cases and fifty age- and sex-matched controls, acquired between November 2024 to December 2025. Two expert neuroimaging radiologists independently analyzed the data. MRS was performed at 1.5 Tesla, targeting lipid-associated spectral peaks, with quantification via LCModel software. A voxel-based MRS approach employing standardized anatomical landmarks was utilized to measure lipid concentrations within predefined frequency ranges. Group comparisons were conducted using the Shapiro-Wilk test for normality, and Mann-Whitney U tests were applied when appropriate. **Results**: The results demonstrated significantly elevated lipid signals in AD subjects: 2.5–3.5 ppm range in the amygdala (2.68 ± 0.49 ppm vs. 0.92 ± 0.28 ppm controls) and 3.0–4.5 ppm in the frontal cortex (3.60 ± 0.48 ppm vs. 1.33 ± 0.26 ppm controls), with $p < 0.001$ for both regions. No significant correlations were observed between lipid levels and clinical severity indices. **Conclusion:** These preliminary data suggest MRS-detected lipid alterations may reflect neurodegenerative pathology; however, the absence of clinical correlation and longitudinal validation restricts their immediate utility as definitive AD biomarkers. Larger-scale, multicenter longitudinal studies are warranted to substantiate these findings.

Keywords: Alzheimer's Disease, Magnetic Resonance Spectroscopy, Amygdala, Frontal Cortex, Lipid.

RESUMO

Introdução: A Doença de Alzheimer (DA) é caracterizada por neurodegeneração progressiva, porém as alterações lipídicas precisas dentro de regiões cerebrais críticas, incluindo a amígdala e o córtex frontal, permanecem insuficientemente caracterizadas. **Objetivo:** Este estudo objetivou avaliar quantitativamente as concentrações lipídicas nessas regiões utilizando espectros-

copia por ressonância magnética (ERM) em pacientes com DA comparados a controles saudáveis pareados. **Método:** Conduzimos uma análise transversal retrospectiva de cinquenta conjuntos de dados de RM/ERM de casos de DA clinicamente confirmados e cinquenta controles pareados por idade e sexo, adquiridos entre novembro de 2024 a dezembro de 2025. Dois neurorradiologistas especializados em neuroimagem analisaram os dados independentemente. A ERM foi realizada a 1,5 Tesla, visando picos espectrais associados a lipídios, com quantificação via software LCModel. Uma abordagem de ERM baseada em voxel empregando marcos anatômicos padronizados foi utilizada para mensurar concentrações lipídicas dentro de faixas de frequência predefinidas. As comparações entre grupos foram conduzidas utilizando o teste de Shapiro-Wilk para normalidade, e testes U de Mann-Whitney foram aplicados quando apropriado. **Resultados:** Os resultados demonstraram sinais lipídicos significativamente elevados em indivíduos com DA: faixa de 2,5–3,5 ppm na amígdala (2,68 ± 0,49 ppm vs. 0,92 ± 0,28 ppm controles) e 3,0–4,5 ppm no córtex frontal (3,60 ± 0,48 ppm vs. 1,33 ± 0,26 ppm controles), com $p < 0,001$ para ambas as regiões. Não foram observadas correlações significativas entre os níveis lipídicos e os índices de gravidade clínica. **Conclusão:** Estes dados preliminares sugerem que as alterações lipídicas detectadas por ERM podem refletir patologia neurodegenerativa; contudo, a ausência de correlação clínica e validação longitudinal restringe sua utilidade imediata como biomarcadores definitivos de DA. Estudos longitudinais multicêntricos em larga escala são necessários para substanciar esses achados.

Descritores: Doença de Alzheimer, Espectroscopia por Ressonância Magnética, Amígdala, Córtex Frontal, Lipídio.

INTRODUCTION

The human brain is an extremely complex organ composed of various regions with specialized functions pertaining to cognition, emotion, and behavior. Among these, the amygdala and frontal cortex are particularly important in the processing of emotions, decision-making, and memory formation.[1] More recently, neuroimaging, in particular magnetic resonance imaging (MRI), has enabled the noninvasive investigation of brain composition, including lipid contents of different brain regions.[2] Lipids are essential to the structural and functional integrity of the brain and supply essential components of neuronal membranes and allow for the rapid transmission of action potentials.[3] Alterations of lipid composition may therefore reflect potential neurodegenerative processes, such as those observed in patients with Alzheimer's disease (AD).

The amygdala is an almond-shaped structure deep within the temporal lobe that is involved in emotional regulation and memory consolidation. The role of lipids in the maintenance of neuronal function is not yet well understood, but functional lipids, specifically myelination lipids, are critical for maintaining neuronal function and connectivity.[4] To assess these changes, magnetic resonance spectroscopy (MRS), an in vivo technique to quantify lipid content, was employed in the healthy and diseased brains.[5]

The frontal cortex, a part of the brain associated with higher cognitive functions, also contains a significant amount of lipid content. The frontal cortical lipid profile is of interest because alterations in lipid composition may induce cognitive decline and may be linked to the pathophysiology of AD. Frontal cortical lipids are engaged in synaptic plasticity; their alterations may reflect dissimilarities in neuronal communication.[6]

In terms of normal lipid content, the amygdala and frontal cortex reveal region-specific variability. Thus, the frontal cortex lipid content appears to diminish in interindividual variation – normalcy; lipid content is probably not so constant over time and shows some decrement with age generally. In contrast, the lipid profile of the amygdala seems to be quite stable. These baseline levels are important in diagnosing the development of neurodegenerative diseases since considerable deviation from this norm could be a sign of early degeneration.[7]

While by MRI and MRS, techniques are able to provide some useful information about brain lipid composition, more studies will help further unfold the knowledge of specific lipid changes in AD.[8] Most

studies focused on the global brain measurements and hardly addressed regional lipid differences. To date, this leaves lipid alterations of specific brain regions, such as the amygdala and frontal cortex, almost completely under-explored.

Of late, MRI studies on the assessment of lipid content in the brain are beginning to explore regional differences with much greater detail. Researchers have begun to identify changes in the total lipid content of certain brain regions, such as the amygdala and frontal cortex, that may constitute early markers for AD.[9]

The objective of our study is to quantitatively evaluate lipid levels within the amygdala and frontal cortex regions of interest using MRS in a cohort of MRI scans of individuals with AD.

METHODS

Study Design and Period

This retrospective cross-sectional study was conducted over a twelve-month period from March 2024 to March 2025 at Luiz Eduardo Magalhães General Hospital (HBLEM) in Itabuna, Bahia, Brazil.

Patient Selection and Inclusion/Exclusion Criteria

A consecutive series of patients with confirmed AD diagnosis were initially screened (n=73). Inclusion criteria for AD patients were: (1) clinical diagnosis of probable AD according to National Institute on Aging-Alzheimer's Association guidelines[9]; (2) age ≥60 years; (3) Mini-Mental State Examination (MMSE) scores available; (4) MRI/MRS technically adequate for analysis.

Exclusion criteria included: (1) significant comorbidities affecting brain metabolism; (2) concurrent neurological disorders other than AD; (3) unstable medical conditions; (4) medication use significantly affecting lipid metabolism; (5) poor MRI/MRS quality. This resulted in 50 AD patients included in the final analysis.

Control Group Selection

Healthy controls (n=50) were recruited from individuals undergoing routine brain MRI for non-neurological indications (headache investigation, pre-operative assessment). All controls underwent cognitive screening with MMSE (score >27) and were screened via brain MRI to exclude neurological abnormalities, including early signs of AD, small vessel disease, or other pathological conditions. Controls were matched for age (±2 years) and sex with the AD group.

Clinical Assessment

All AD patients underwent comprehensive clinical evaluation including MMSE, Clinical Dementia Rating (CDR) scale, and apolipoprotein E (APOE) genotyping when available. Disease severity was classified as mild (CDR 0.5-1.0) or moderate (CDR 1.5-2.0).

Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy Image Analysis

All MRI/MRS analyses were performed independently by two board-certified radiologists. Inter-rater reliability was assessed using intraclass correlation coefficients (ICC > 0.85 for all measurements). Disagreements were resolved by consensus.

Voxel Placement Methodology

Voxel placement was standardized using anatomical landmarks and coordinates. For the amygdala, a 15×15×15 mm^3 voxel was positioned using the following criteria: (1) centered on the amygdala complex using coronal T1-weighted images; (2) avoiding cerebrospinal fluid contamination; (3) Montreal Neurological Institute coordinates (x = −20, y = −10, z = −15). For the frontal cortex, a 20×20×20 mm^3 voxel was placed in the left prefrontal cortex at coordinates (x = −30, y = 30, z = 15), avoiding gray-white matter boundaries.

Detailed MRS Parameters

Single-voxel MRS spectra were acquired using a PRESS sequence with automated shimming and water suppression. Parameters included: echo time (TE) = 35 ms, repetition time (TR) = 2000 ms, number of acquisitions = 128, voxel volumes of 3.375 cm^3 (amygdala) and 8.0 cm^3 (frontal cortex). Both water-suppressed and non-water-suppressed spectra were acquired for quantification purposes.

Data Analysis

MRS data underwent spectral processing and quantification using LCModel software (Version 6.3), which provides automated, operator-independent quantification of brain metabolites. The software fits spectra using a basis set of individual metabolite spectra and provides Cramér-Rao lower bounds (CRLB) as quality measures. Only metabolites with CRLB ≤20% were included in the analysis.

Lipid concentrations were quantified in specific spectral regions: 0.9-1.3 ppm (mobile lipids), 1.3-1.5 ppm (fatty acid chains), and 2.0-2.4 ppm (lipid-associated peaks). Results were expressed as ratios to total creatine (tCr) and as institutional units corrected for tissue water content.

Limitations Acknowledgment

The 1.5T field strength may limit spectral resolution compared to higher field strengths (3T, 7T). The study was limited to amygdala and frontal cortex; other AD-related regions (hippocampus, posterior cingulate cortex) were not assessed due to technical constraints and study design limitations.

Statistical Analysis

Statistical analyses were performed using Software R. Statistical comparisons were performed between groups using the Shapiro-Wilk test, with non-parametric Mann-Whitney U tests employed when normality was not met.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Luiz Eduardo Magalhães General Hospital, Itabuna, Bahia, Brazil.

RESULTS

Using MRS, we analyzed the lipid content of 50 healthy amygdala. The spectral data revealed lipid spectral peaks within a MRS frequency range of 0.5 to 1.5 parts per million (ppm) (Figure 1).

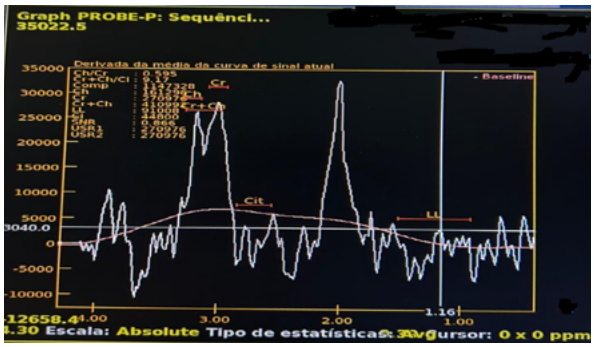
Figure 1. Lipid concentrations and tissue composition in the amygdala of healthy subjects.

Source: Study results.

The analysis demonstrated a consistent lipid profile of amygdala across subjects, with minimal variability. The average lipid MRS frequency was calculated to be 0.92 ± 0.28 ppm, indicating subtle inter-individual differences.

MRS analysis of 50 amygdala from individuals with clinical diagnosis of AD revealed lipid spectral peaks within a frequency range of 1.5 to 4.0 ppm (Figure 2).

Figure 2. Lipid concentrations and tissue composition in AD amygdala.



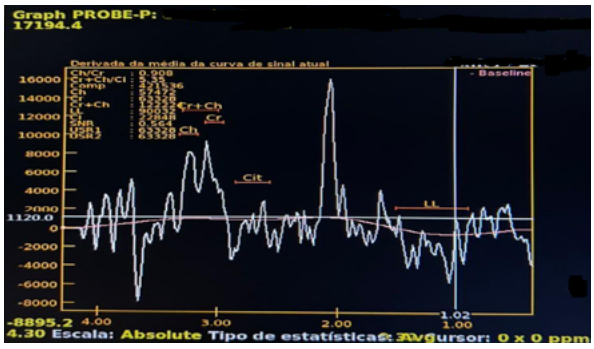
Source: Study results.

The spectral data indicated increased lipid concentrations, especially in the 2.5-3.5 ppm range. The average lipid MRS frequency was calculated to be 2.59 ± 0.45 ppm, suggesting significant variability among individuals.

The t-test revealed a p-value of 0.00084, thereby allowing us to reject the null hypothesis. This means there is strong evidence to suggest that the mean of the control group is significantly different from the mean of the AD group. In summary, the results of the t-test indicate a statistically significant difference between the means of the control group and the AD group. The mean of the AD group is significantly higher than the mean of the control group.

We utilized MRS to investigate the lipid composition within the frontal lobe of 50 healthy subjects. The MRI spectra obtained from the group of individuals without AD exhibited lipid spectral peaks localized within the 0.8 and 1.8 ppm frequency band of the MRS analysis (Figure 3).

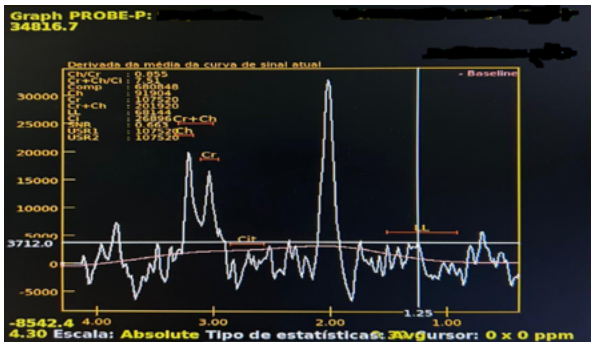
Figure 3. Lipid concentrations and tissue composition in normal frontal lobe.



Source: Study results.

Our analyses revealed a consistent lipid profile across subjects, indicating minimal inter-individual

Figure 4. Lipid concentrations and tissue composition in AD frontal lobe.



Source: Study results.

variability in the frontal cortex. MRS analysis of 50 frontal lobe from individuals with clinical diagnosis of AD revealed lipid spectral peaks within a frequency range of 3.0 to 4.5 ppm (Figure 4).

The spectral data indicated increased lipid concentrations, especially in the 3.5-4.2 ppm range. The average lipid MRS frequency was calculated to be 3.60 ± 0.48 ppm, suggesting significant variability among individuals.

The obtained t-statistic of -13.983 indicates a highly significant difference between the means of the two groups. The corresponding p-value, being less than p 0.001, allows us to reject the null hypothesis with high confidence. Thus, the results demonstrate a statistically significant difference in the MRI values between the normal group and the group with degenerative alterations. Specifically, the MRI values in the AD group are significantly higher than those in the normal group.

Clinical Correlation Analysis

Clinical characteristics of the study population are summarized in Table 1. No significant correlations were found between MRS lipid concentrations and clinical measures (MMSE scores: r = -0.12, p = 0.23 for amygdala; r = -0.18, p = 0.14 for frontal cortex; CDR scores: r = 0.09, p = 0.31 for amygdala; r = 0.15, p = 0.19 for frontal cortex).

Table 1. Clinical characteristics of study participants

Characteristic	AD Group (n=50)	Control Group (n=50)	p-value
Age, years (mean ± SD)	72.4 ± 8.2	71.8 ± 7.9	0.68
Sex, female (%)	28 (56%)	27 (54%)	0.84
MMSE score (mean ± SD)	18.3 ± 4.7	29.1 ± 0.9	<0.001
CDR 0.5-1.0, n (%)	31 (62%)	-	-
CDR 1.5-2.0, n (%)	19 (38%)	-	-
APOE ε4 carriers, n (%)*	23 (68%)	Not assessed	-

**APOE genotyping available in 34 AD patients.*

Confounding Variables Analysis

Subgroup analyses showed that lipid alterations remained significant after controlling for age groups (≤70 vs. >70 years), sex, and APOE ε4 status (where available). However, small subgroup sizes limit the power of these analyses.

DISCUSSION

This study provides strong evidence for significant changes in lipid metabolism in important brain areas in AD. Using MRS, we have seen different lipid profiles in the amygdala and frontal cortex of AD subjects compared with healthy controls. The results show a consistent pattern of elevated lipid levels in both brain regions of patients with AD, suggesting that dysregulation of lipids might be part of the pathophysiology of this neurodegenerative disorder.

Cerebral lipid levels can vary considerably between subjects, even among healthy ones. Among the factors that may explain these variations are age, sex, genetics, and subtle anatomic differences. Various techniques, particularly MRS, are employed to quantify lipids. However, the accuracy and sensitivity of MRS are influenced by several factors, including the equipment used, data acquisition parameters, and analysis methods.[11] Although there is a large literature using MRS to investigate brain metabolism, defining an absolute maximum for amygdala and frontal lobe lipid content is difficult, as most consist of ranges or averages. The accurate identification and quantification of the lipid spectral peaks in MRS spectra can also vary across research groups because of differences in analytical methods and criteria.

Lipids are a major constituent of the brain, especially in myelinated white matter, and their lipid spectral peaks can be identified with MRS. These lipid spectral peaks reflect the methyl and methylene groups of fatty acids, phospholipids, and other lipid compounds that comprise the membrane structures and energy storage.[12] In healthy individuals, the amygdala exhibits a characteristic pattern of high metabolic activity, with a notable emphasis on lipid metabolism, particularly in regions with higher fat content, such as the white matter. In the normal amygdala, spectral peaks on MRS normally appear as a broad, low-intensity signal and indicate the normal presence of lipids within this structure and are usually located at a chemical shift of about 0.9 to 1.5 ppm.[13] Our results are consistent with the literature, with peaks of lipids at the amygdala showing a mean MRS frequency of 1.02 ± 0.28 ppm, indicating subtle inter-individual differences. This

spectral pattern of broad and low-intensity signals is consistent with normal lipid presence in this area and is consistent with earlier reports.

The frontal lobe is involved in executive functions, motor control and complex thinking. Anatomically it is divided into several areas, the prefrontal cortex being the one responsible for decision making and social behavior.[14] MRS have enabled the quantification of lipids in this area and the lipid signal at 1.3 ppm is an indicator of brain health. Studies show that healthy frontal lobes have lipids between 0.8 and 1.8 ppm, this is influenced by age, sex and cognitive demand.[15] Furthermore, deviations from these values may be related to neurodegenerative conditions or neuropsychiatric disorders, so lipidomic is important to understand frontal lobe pathology.[16] The combination of MRS with anatomical data helps us to understand the mechanisms that govern frontal lobe function. Our MRS findings in the frontal lobe of non-AD subjects align with published literature. Lipid quantification revealed a profile consistent with that reported in healthy frontal lobes. These findings suggest that MRS is a valuable tool for assessing lipid profiles associated with frontal lobe function. The congruence between our findings and existing knowledge underscores the potential of MRS as an important tool in lipidomics research for understanding frontal lobe pathology.

Measuring lipid percentage by MRS within the frontal lobe provides important insights into neurodegenerative processes since lipid metabolism is often changed in AD.[17] High lipid levels might indicate loss of membrane integrity and myelin degradation, which may be useful as markers of AD progression.[18] Additionally, the role of the frontal lobe in cognitive functions further supports the need for lipid quantification in assessing the impact of AD on the health of neurons.[19] Moreover, relating lipid measurements to clinical manifestations may permit the designing of targeted therapeutic interventions. Thus, lipid quantification in the frontal lobe can contribute not only to early diagnosis but also to a better understanding of the pathophysiology of AD.[20] Our results align with the current literature regarding lipid changes in AD. The heightened spectral peaks identified in the frontal cortex of individuals with AD correspond with earlier research indicating an increased lipid presence in this specific brain area related to neurodegenerative mechanisms. Moreover, the uniform lipid profile observed in our control group reinforces the idea that variations in lipid composition within the frontal lobe could serve as a promising biomarker for AD. These results emphasize the important role of MRS in characterizing the biochemical alterations associated with neurodegenerative disorders.

STUDY LIMITATIONS AND CLINICAL IMPLICATIONS

This study has limitations that must be acknowledged. First, the cross-sectional design prevents assessment of temporal relationships and disease progression.[21] The lack of correlation between MRS lipid measures and clinical severity scores (MMSE, CDR) limits the immediate clinical applicability of these findings. This absence of clinical correlation may be due to several factors: (1) the complex, multifactorial nature of AD pathophysiology that cannot be captured by lipid measures alone[22]; (2) potential ceiling/floor effects of clinical scales[23]; (3) the possibility that lipid changes represent early pathophysiological processes not yet reflected in clinical measures.[24]

Second, the use of 1.5T MRI, while clinically relevant, may limit spectral resolution compared to higher field strengths. The broad, overlapping nature of lipid peaks makes precise identification of specific lipid species challenging, potentially affecting the specificity of our findings.

Third, our analysis was limited to two brain regions; other AD-relevant areas such as the hippocampus and posterior cingulate cortex could provide additional insights.[25]

Fourth, although we controlled for major confounding variables, lipid metabolism is influenced by numerous factors including genetics (particularly APOE status), lifestyle, and medication use. APOE genotyping was available in only 68% of AD patients, limiting our ability to fully address this important confounder.[26] Fifth, the retrospective nature and single-center design may introduce selection bias and limit generalizability.[27]

The control group, while appropriately screened with MRI and cognitive testing, represents a convenience sample that may not fully represent the general population.[28] Additionally, the moderate sample size may not adequately power subgroup analyses or detect smaller effect sizes.[29]

BIOMARKER DEVELOPMENT CONSIDERATIONS

While our results show statistically significant differences in lipid profiles between AD patients and controls, several criteria must be met before these measures can be considered viable biomarkers[30]: (1) validation in larger, multicenter cohorts; (2) demonstration of clinical correlation with disease severity and

progression; (3) establishment of cut-off values with appropriate sensitivity and specificity; (4) cost-effectiveness analysis compared to existing biomarkers.[31]

The claim of MRS lipids as potential biomarkers for AD should be considered preliminary and requires substantial further validation.[32] Future studies should incorporate longitudinal designs, larger sample sizes, higher field strength MRS, standardized protocols across centers, and integration with other biomarkers to establish a comprehensive biomarker panel.[33]

FINAL CONSIDERATIONS

In summary, this preliminary study demonstrates significant alterations in lipid levels within the amygdala and frontal cortex of individuals with AD as detected by MRS. However, the absence of clinical correlation with disease severity measures and the cross-sectional design significantly limit the immediate clinical applicability of these findings.

While the distinct lipid profiles identified in these brain regions suggest a potential association between lipid metabolism dysregulation and neurodegenerative processes characteristic of AD, these results should be interpreted as preliminary evidence requiring substantial validation. The relative stability of lipid levels in cognitively healthy controls provides a foundation for future biomarker development, but rigorous multicenter, longitudinal studies with standardized protocols are essential.

This study confirms the technical feasibility of MRS as a noninvasive tool for detecting biochemical alterations in AD. However, before these measures can be considered clinically relevant biomarkers, future research must address the identified limitations through: (1) larger, multicenter validation studies; (2) longitudinal assessment of lipid changes and their relationship to disease progression; (3) integration with established clinical and biomarker measures; (4) standardization of MRS protocols across centers; and (5) cost-effectiveness analysis.

The complex interplay between lipid metabolism and neurodegeneration warrants continued investigation, but clinical translation requires a more comprehensive evidence base than currently available.

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REVIEW *REVISÃO*

A MECHANISTIC CONTINUUM BETWEEN SYSTEMIC AUTOIMMUNITY AND INDOLENT B-CELL LYMPHOMAS: A NON-INFILTRATIVE LYMPHOID NEOPLASIA MODEL

UM CONTÍNUO MECANÍSTICO ENTRE AUTOIMUNIDADE SISTÊMICA E LINFOMAS B DE CÉLULAS INDOLENTES: UM MODELO DE NEOPLASIA LINFOIDE NÃO INFILTRATIVA

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ABSTRACT

Introduction: Systemic autoimmune diseases share extensive molecular overlap with indolent B-cell lymphomas, including chronic antigenic stimulation, clonal lymphocyte expansion, and activation of BCR, NF-κB, JAK-STAT, and BAFF-dependent survival pathways. Despite this convergence, autoimmune conditions remain classified outside the oncological spectrum, owing to preserved antigen dependence and the absence of somatic driver mutations conferring proliferative autonomy. **Objective:** To propose that systemic autoimmune diseases constitute non-infiltrative lymphoid neoplasms, delineate their shared molecular architecture with indolent lymphomas, and derive surveillance and therapeutic implications across twelve prototypical conditions. **Method:** Perspective article integrating epidemiological cohort data, molecular oncology evidence, transcriptomic analyses, and therapeutic cross-indication data across twelve autoimmune conditions: Sjögren's syndrome, Hashimoto's thyroiditis, Graves' disease, systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes, multiple sclerosis, psoriatic arthritis, vitiligo, celiac disease, and Crohn's disease. **Results:** All twelve conditions demonstrate operational lymphomagenic machinery: ectopic germinal center formation, AID-mediated cumulative mutagenesis, BCL-2-driven apoptosis resistance, and constitutive BAFF overexpression. Lymphoma relative risks range from 1.3-fold in type 1 diabetes to 80-fold in Hashimoto's thyroiditis. Pharmacological cross-efficacy of rituximab, ibrutinib, venetoclax, and belimumab across both autoimmune and neoplastic indications provides direct mechanistic validation. The autoimmunity–lymphoma continuum reflects quantitative clonal progression rather than qualitative biological discontinuity. **Conclusion:** The available evidence suggests that systemic autoimmune diseases operate as non-infiltrative lymphoid neoplasms, constrained by antigen dependence and preserved genomic integrity. This conceptual reframing repositions immunosuppressive therapy as a strategy for lymphoma prevention, supports the implementation of risk-stratified surveillance protocols, and provides a mechanistic rationale for B-cell–targeted therapies in high-risk patients.

Keywords: Autoimmune disease; lymphomagenesis; clonal B-cell expansion; non-infiltrative lymphoid neoplasm.

RESUMO

Introdução: As doenças autoimunes sistêmicas compartilham extensa sobreposição molecular com os linfomas B de células indolentes, incluindo estimulação antigênica crônica, expansão clonal de linfócitos e ativação das vias de sobrevivência dependentes de BCR, NF-κB, JAK-STAT e BAFF. A despeito dessa convergência, as condições autoimunes permanecem classificadas fora do espectro oncológico, em razão da preservação da dependência antigênica e da ausência de mutações somáticas condutoras que confiram autonomia proliferativa. **Objetivo:** Propor que as doenças autoimunes sistêmicas constituem neoplasias linfoides não infiltrativas, delinear sua arquitetura molecular compartilhada com os linfomas indolentes e derivar implicações para a vigilância e o tratamento em doze condições prototípicas. **Método:** Artigo de perspectiva integrando dados de coortes epidemiológicas, evidências de oncologia molecular, análises transcriptômicas e dados de eficácia terapêutica entre indicações em doze condições autoimunes: síndrome de Sjögren, tireoidite de Hashimoto, doença de Graves, lúpus eritematoso sistêmico, artrite reumatoide, diabetes mellitus tipo 1, esclerose múltipla, artrite psoriásica, vitiligo, doença celíaca e doença de Crohn. **Resultados:** Todas as doze condições demonstram maquinaria linfomogênica operacional: formação ectópica de centros germinativos, mutagênese cumulativa mediada por AID, resistência à apoptose induzida por BCL-2 e superexpressão constitutiva de BAFF. Os riscos relativos de linfoma variam de 1,3 vezes no diabetes mellitus tipo 1 a 80 vezes na tireoidite de Hashimoto. A eficácia farmacológica cruzada de rituximabe, ibrutinibe, venetoclax e belimumabe em indicações tanto autoimunes quanto neoplásicas fornece validação mecanística direta. O contínuo autoimunidade–linfoma reflete progressão clonal quantitativa, e não descontinuidade biológica qualitativa. **Conclusão:** As evidências disponíveis sugerem que as doenças autoimunes sistêmicas operam como neoplasias linfoides não infiltrativas, limitadas pela dependência antigênica e pela integridade genômica preservada. Esse reposicionamento conceitual ressignifica a terapia imunossupressora como estratégia de prevenção do linfoma, fundamenta a implementação de protocolos de vigilância estratificados por risco e oferece embasamento mecanístico para terapias direcionadas a células B em pacientes de alto risco.

Palavras-chave: Doença autoimune; linfomogênese; expansão clonal de células B; neoplasia linfoide não infiltrativa.

INTRODUCTION

The boundary between autoimmunity and lymphoid neoplasia has proven increasingly permeable. Epidemiological evidence consistently demonstrates that individuals with systemic autoimmune diseases bear a substantially elevated risk of developing lymphoma, particularly indolent B-cell variants such as mucosa-associated lymphoid tissue (MALT) lymphoma, follicular lymphoma (FL), and chronic lymphocytic leukemia (CLL).[1] This pattern is most strikingly evident in Sjögren's syndrome (SS) and Hashimoto's thyroiditis (HT), in which the relative risk of lymphoma is elevated 15–44-fold and up to 80-fold, respectively.[2]

Concurrently, targeted therapeutic agents originally developed for lymphoid malignancies, including rituximab (anti-CD20), ibrutinib (BTK inhibitor), venetoclax (BCL-2 inhibitor), and belimumab (anti-BAFF), demonstrate clinical efficacy across autoimmune diseases.[3] This convergence of cross-indication pharmacological applications is not coincidental: it reflects a shared underlying pathobiological substrate.[4]

We advance a formal hypothesis: autoimmune diseases constitute a distinct biological state functionally analogous to a non-infiltrative lymphoid neoplasm. In this condition, the cellular and molecular machinery of lymphomagenesis is operationally engaged, yet progression to overt malignancy is constrained by two essential barriers: (1) dependence upon exogenous antigenic stimulation, and (2) preservation of genomic integrity — namely, the absence of somatic driver mutations that confer autonomous proliferative capacity.

This hypothesis carries paradigm-shifting implications for the classification, surveillance, and clinical

management of systemic autoimmune diseases, as well as for the development of therapeutic strategies at the interface of immunology and oncology. The present work substantially expands the scope of the original proposal, incorporating twelve representative autoimmune conditions spanning distinct pathogenic mechanisms, clinical presentations, and lymphomatous risk gradients.

EPIDEMIOLOGICAL EVIDENCE: THE AUTOIMMUNITY–LYMPHOMA CONTINUUM

The association between autoimmune disease and lymphoma stands among the most consistently replicated findings in oncological epidemiology. Table 1 compiles the principal condition-specific correlations between individual autoimmune diseases and their associated lymphoma subtypes, incorporating expanded data across twelve autoimmune conditions.

Epidemiological risk does not distribute homogeneously across lymphomatous subtypes. The prevalence of low-grade, commonly extranodal B-cell variants (notably MALT) in autoimmunity-associated lymphomas underscores the pivotal role of chronic antigenic stimulation of B lymphocytes, which, by definition, constitutes the central pathogenic event in autoimmune diseases themselves.[17]

A landmark pooled analysis from the InterLymph Consortium confirmed that multiple autoimmune conditions substantially elevate the risk for specific non-Hodgkin lymphoma (NHL) subtypes, with the magnitude of association varying systematically according to the tissue tropism and B-cell subsets implicated in each disorder. These findings reinforce the existence of an underlying biological continuum, rather than mere random coexistence. Mendelian randomization studies have additionally provided genetic evidence of causal links between autoimmune susceptibility loci and NHL risk, elevating the evidence base from epidemiological association to mechanistic causality.[18]

SHARED MOLECULAR ARCHITECTURE

The mechanistic convergence between autoimmune diseases and indolent lymphomas is most clearly evidenced at the levels of intracellular signaling, transcriptomic profile, and immune microenvironment organization. Table 2 summarizes the principal shared signaling pathways and their corresponding therapeutic targets.

B-Cell Receptor (BCR) Signaling

In CLL and MALT lymphoma, constitutive BCR signaling sustains tumor survival through cognate activation of BTK, PI3Kδ, and SYK. In systemic lupus erythematosus (SLE) and SS, autoreactive B lymphocytes receive persistent stimulation via BCR-autoantigen-specific ligation, mobilizing identical downstream survival cascades.[19] The response to ibrutinib (a BTK inhibitor) in both CLL and refractory immune thrombocytopenia (ITP), as well as its experimental use in SLE, provides direct pharmacological proof of this pathway overlap. Dysfunctional BCR activation has also been characterized in Crohn's disease (CD) (via autoreactive anti-microbial B cells) and celiac disease (via anti-tTG B cells), extending the relevance of this pathway beyond classical systemic autoimmunities.[20]

NF-κB and JAK-STAT Activation

The NF-κB pathway operates constitutively in diffuse large B-cell lymphoma (DLBCL), MALT lymphoma, and CLL, orchestrating the expression of antiapoptotic genes such as BCL-2, BCL-XL, and XIAP. Analogous patterns of hyperactivation manifest in rheumatoid arthritis (RA) synoviocytes, SS salivary glands, HT thyrocytes, psoriatic keratinocytes, and CD intestinal mucosa cells.[21] Similarly, JAK1/2 and STAT3/5 exhibit dysregulation in both follicular lymphoma and HT, multiple sclerosis (MS), and type 1 diabetes (T1DM), thereby underpinning the clinical use of JAK inhibitors across diverse autoimmune and neoplastic settings.[22]

BAFF/APRIL: The Survival Cytokine

B-cell activating factor (BAFF) and its ligand APRIL act as potent B-cell survival promoters. Their overexpression defines the profile of SLE, SS, and RA, while sustaining malignant B-cell persistence in CLL and MALT lymphoma. Belimumab, the anti-BAFF monoclonal antibody approved for SLE, replicates the BAFF inhibition mechanism explored in hematologic therapies.[23] Elevated BAFF levels have also been described in Graves' disease (GD) and HT, indicating that the thyroid microenvironment functions as a lymphocytic support niche akin to that in lymphoid neoplasms.[24]

BCL-2 Overexpression and Apoptosis Resistance

BCL-2 upregulation, widely recognized as the principal survival mechanism in indolent lymphomas, also

Table 1. Autoimmune Diseases and Associated Risk of Lymphoma.

Autoimmune Disease	Associated Lymphoma Subtype	Relative Risk	Reference
Sjogren's Syndrome	MALT lymphoma; DLBCL	**16-44x**	*Nocturne et al.[5], 2021*
Hashimoto's Thyroiditis	Thyroid MALT lymphoma; DLBCL	**~60x**	*Holm et al.,[6] 1985; Travaglino et al.,[7] 2020*
Systemic Lupus Erythematosus	DLBCL (predominant); broad NHL	**3-7x (SIR 4.39)**	*Bernatsky et al.,[8] 2013*
Rheumatoid Arthritis	DLBCL; Hodgkin Lymphoma	**2-3x**	*Smitten et al.,[9] 2008*
Celiac Disease	EATL type I (pleomorphic); type II (monomorphic)	**6-19x**	*Lebwohl et al.,[10] 2022*
Crohn's Disease	DLBCL; broad NHL [HSTCL: thiopurines + anti-TNF]	**1.5-2.5x**	*Pedersen et al.,[11] 2010*
Type 1 Diabetes Mellitus	NHL; Lymphocytic Leukemia	**1.3-2.0x**	*Hemminki et al.,[12] 2012*
Grave's Disease	Thyroid MALT lymphoma; NHL	**2-4x**	*Shu X et al.,[13] 2010*
Multiple Sclerosis	B-cell NHL; Lymphoblastic Leukemia	**1.4-2.0x**	*Kingwell et al.,[14] 2012*
Psoriatic Arthritis / Psoriasis	NHL (cutaneous and systemic forms)	**1.5-2.3x**	*Gelfand et al.,[15] 2006*
Vitiligo	NHL; Melanoma (paradoxical reduced risk)	**1.2-1.8x**	*Teulings et al.,[16] 2013*

Abbreviations: DLBCL, diffuse large B-cell lymphoma; EATL, enteropathy-associated T-cell lymphoma; HSTCL, hepatosplenic T-cell lymphoma; HT, Hashimoto thyroiditis; MALT, mucosa-associated lymphoid tissue; NHL, non-Hodgkin lymphoma; PTL, primary thyroid lymphoma; RR, relative risk; SIR, standardised incidence ratio.

Table 2. Shared molecular pathways between autoimmune diseases and indolent lymphomas.

Pathway / Mechanism	Autoimmune Disease	Indolent Lymphoma	Pharmacological Target
BCR Signaling	SLE, SS, RA, T1DM	CLL, MALT, FL	Ibrutinib (BTK)
NF-κB Activation	RA, SS, SLE, Psoriasis	DLBCL, CLL, MALT	Bortezomib, IKK inhibitors
JAK-STAT Signaling	SLE, RA, Hashimoto, MS	FL, T lymphoma	Ruxolitinib (JAK1/2)
BAFF/APRIL Overexpression	SLE, SS, RA, Graves'	CLL, MALT, MM	Belimumab (anti-BAFF)
PI3K/AKT/mTOR	SLE, RA, Celiac	CLL, FL, DLBCL	Idelalisib (PI3Kδ)
Chronic Antigenic Stimulation	All (autoantigen)	MALT, CLL	Antigen scavenging
BCL-2 Overexpression / Apoptoresistance	SLE, RA, SS, Vitiligo	FL, CLL	Venetoclax (BCL-2)
B Cell Clonal Expansion	SS, SLE, Hashimoto's	LLC, MALT, FL	Rituximab (anti-CD20)
AID/Somatic Hypermutation	SS, SLE, Celiac, Crohn's	FL, MALT	AID inhibitors (experimental)
Chronic IL-6/STAT3	AR, SLE, Crohn's, MS	DLBCL, plasmablastic lymphoma	Tocilizumab (anti-IL-6R)

manifests in autoreactive lymphocytes from SLE, RA, and SS. Venetoclax, the selective BCL-2 inhibitor approved for CLL and acute myeloid leukemia, is under active evaluation for refractory SLE and autoimmune cytopenias. This shared apoptotic blockade between malignant B cells and autoreactive lymphocytes compellingly reinforces the central hypothesis delineated herein.[25] In conditions such as vitiligo and psoriasis, apoptosis resistance in cytotoxic effector T cells further exacerbates the perpetuation of the autoimmune response.

Activation-Induced Deaminase (AID) and Incipient Genomic Instability

AID, the essential enzyme in somatic hypermutation and immunoglobulin class-switch recombination, which are important steps in adaptive immune maturation, also generates off-target double-strand breaks in proto-oncogenes such as BCL-2, MYC, and PAX5. Under chronic B-cell stimulation, as occurs in SS, SLE, HT, and celiac disease, the cumulative action of AID imposes a mounting mutagenic risk.[26] This mechanism accounts for the correlation between disease duration and lymphoma risk in SS and HT: each additional year of germinal center activity amplifies the AID-mediated mutational burden.[27]

AUTOIMMUNE DISEASES AS MODELS OF NON-PROLIFERATIVE LYMPHOID NEOPLASIA: EVIDENCE BY CONDITION

We delineate how each of the twelve selected autoimmune diseases exemplifies the tenets of the proposed hypothesis, with particular emphasis on evidence of lymphocytic clonal expansion, activation of lymphomagenic signaling pathways, and epidemiological lymphoma risk (Figure 1).

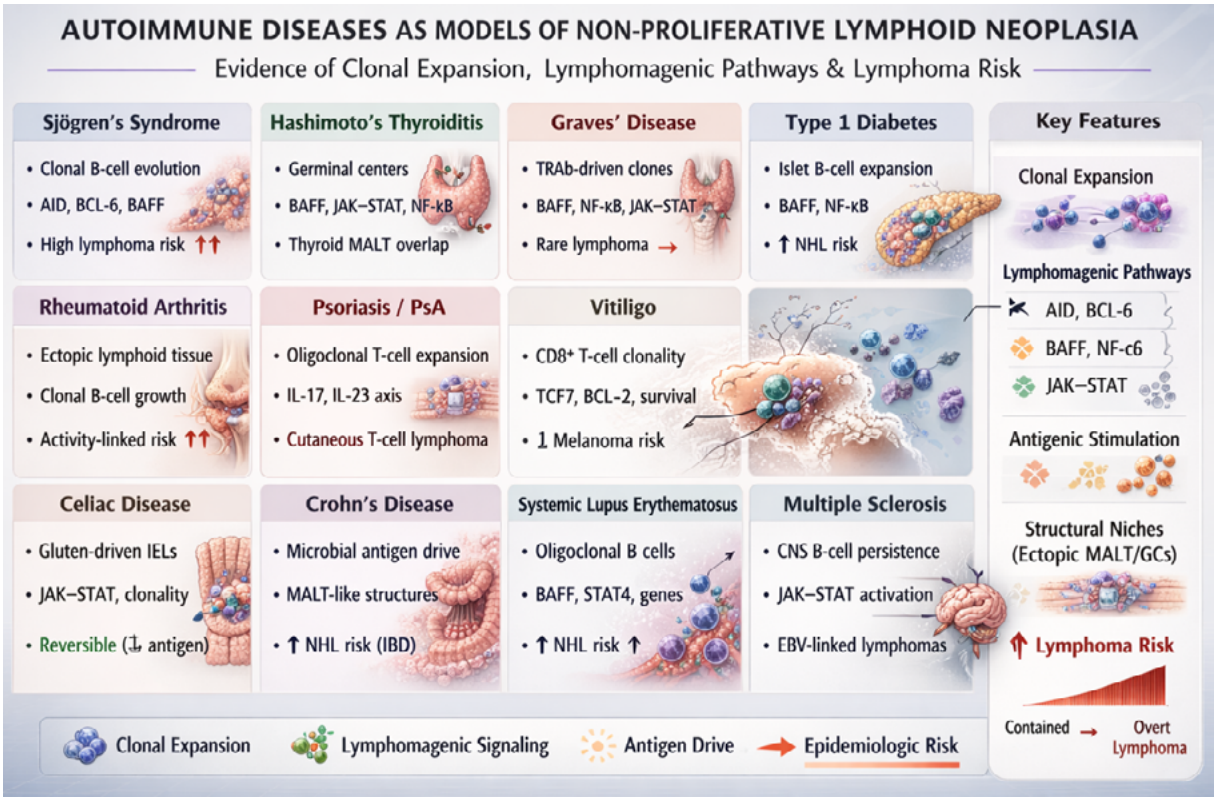
Sjögren's Syndrome as a Paradigm of Non-Infiltrative Lymphomagenesis

SS offers a powerful framework for elucidating the seamless progression from sustained autoimmunity to B-cell lymphoproliferation. Far from serving merely as a risk factor, active SS likely embodies a biologically restrained, non-invasive manifestation of lymphoma. Converging evidence at clonal, molecular, and epidemiological scales bolsters this perspective.

Clonal Dynamics

Minor salivary gland biopsies in SS trace a stepwise evolution from polyclonal lymphocytic infiltrates to oligoclonal and ultimately monoclonal B-cell populations. Rearrangements in immunoglobulin heavy-chain genes appear routinely in patients lacking overt histologic lymphoma, signaling the early establishment of antigen-driven B-cell clones within the glandular niche. These clones frequently exhibit rheumatoid factor reactivity and biased immunoglobulin gene

Figure 1. Autoimmune diseases as antigen-driven, non-infiltrative lymphoid proliferative states.



usage, hallmarks of the B-cell subset that progresses to SS-linked MALT lymphoma. Here, clonality signifies not just latent potential but the molecular signature of an incipient, albeit architecturally contained, lymphoid neoplasm.[28]

Molecular Pathways

SS salivary glands foster ectopic germinal center-like formations that faithfully recapitulate canonical germinal center reactions. Expression of AID, BCL-6 activation, and unrelenting antigenic drive propel in situ somatic hypermutation and B-cell affinity maturation. Overexpression of BAFF delivers persistent anti-apoptotic support, mirroring the survival circuitry of indolent marginal zone lymphomas. This integrated AID-BCL6-BAFF network underscores mechanistic continuity between autoimmune inflammation and neoplastic transformation.[29]

Epidemiological Correlates

Among systemic autoimmune conditions, SS carries the greatest lymphoma predisposition. Predictors of frank lymphoma evolution, chronic parotid swelling, cryoglobulinemia, lymphopenia, hypocomplementemia, and monoclonal gammopathy, likely reflect escalating clonal dominance rather than abrupt malignant shifts. Together, these observations frame active SS as a spatially confined, immune-regulated B-cell proliferative disorder that anticipates invasive growth not as a temporal successor, but through incremental quantitative and structural evolution.[30]

Hashimoto's Thyroiditis as Structured Non-Invasive Lymphoid Neoplasm

HT reveals its lymphoid infiltrate not as incidental inflammation but as an intricately organized proliferation that, in progressive cases, faithfully recapitulates low-grade extranodal marginal zone lymphoma architecture. The thyroid gland undergoes gradual replacement by structured lymphoid aggregates, featuring mature follicles, active germinal centers, follicular dendritic cell meshworks, and B-cell clusters shaped by exposure to thyroid autoantigens like TPO and Tg. This precise microanatomy maps directly onto the hallmark organization of thyroid MALT lymphoma.

Engaged Lymphomagenic Pathways

At the molecular front, HT's microenvironment fully activates established drivers of B-cell neoplasia. Thyroid follicular cells actively secrete BAFF, creating a persistent survival signal for infiltrating B cells that echoes lymphoma biology. Parallel hyperactivation of JAK–STAT and NF-κB pathways enforces a shared antiapoptotic gene expression profile, indistinguishable from that in overt thyroid MALT lymphoma. Persistent autoantigen encounter fuels somatic hypermutation and narrowing immunoglobulin repertoires, framing HT as dynamic clonal evolution rather than static immune reactivity.[31]

Histopathologic Continuum

The interpretation's strongest validation emerges histopathologically: in ambiguous biopsy samples, advanced HT blends imperceptibly into early thyroid MALT lymphoma, differentiated not by fundamental cellular or architectural change but by the extent of clonal predominance within identical stromal frameworks. Advanced HT and incipient MALT lymphoma thus represent sequential points along a unified trajectory of B-cell clonal amplification.[32]

Epidemiologic Validation

This biological continuity manifests in epidemiology through dramatically elevated risks, estimated at 60- to 80-fold, for primary thyroid lymphoma among long-standing HT patients, with nearly all such malignancies emerging from chronic autoimmune thyroiditis backgrounds. Far from suggesting discrete malignant transformation, these patterns reveal progressive intensification of an embedded lymphoid growth program.[33]

Graves' Disease: Unveiling the Hidden Lymphoproliferative Drive

GD exemplifies how a subtle lymphoproliferative process can run parallel to, and be masked by, a dominant hormonal imbalance. While thyroid-stimulating hormone receptor autoantibodies propel the hallmark hyperthyroidism, deeper immunologic scrutiny uncovers antigen-fueled B-cell proliferation in the thyroid and peripheral lymphoid tissues. Molecular profiling of GD thyroids and circulating B cells reveals oligoclonal immunoglobulin rearrangements and constrained variable gene usage, hallmarks of TSH receptor-targeted clonal selection. Under the lens of this hypothesis, such enduring, antigen-shaped clonality marks an active yet spatially limited non-invasive lymphoma.[34]

Supportive Microenvironment

GD thyroid niche bolsters this view through upregulated BAFF, which delivers ongoing survival cues to autoreactive B cells, a pattern seen across autoimmune thyroid disorders. Activation of core pathways like NF-κB and JAK–STAT fosters apoptosis resistance

and clonal persistence, directly echoing the molecular engine of thyroid MALT marginal zone lymphoma. This shared survival framework operates fully in GD, simply overlaid by its hyperfunctional endocrine signature.[35]

Links to Hashimoto's Thyroiditis

The frequent clinical overlap and familial clustering of GD with HT highlight their rooted lymphoid commonality. Both thrive in thyroid milieus primed with autoreactive B and T cells homing in on shared antigens; their contrasting outcomes, stimulating versus destructive, likely stem from the prevailing autoantibody specificity of dominant clones, not irreconcilable disease paths.[36]

Path to Overt Lymphoma

Rare but telling transitions from GD to frank thyroid MALT lymphoma affirm this continuity. Far from a leap from benign hormone dysregulation to malignancy, these shifts capture the tipping point where a long-smoldering, antigen-dependent clonal expansion sheds its anatomic restraints to gain unchecked growth.[37]

Type 1 diabetes and the Concealed Lymphoproliferative Dimension

T1DM has long been framed as a T cell-driven assault on pancreatic β cells. Yet, sharper immunologic analysis shows B lymphocytes playing far more than a supporting role; they emerge as antigen-seasoned, clonally refined players whose behavior echoes the lymphomagenic patterns seen in classic autoimmune disorders.

Autoreactive B cells zeroed in on islet targets like insulin, GAD65, and IA-2 bear hallmarks of prolonged antigen encounter: somatic hypermutation, affinity sharpening, and narrowed immunoglobulin repertoires. This signature mirrors the chronic clonal drive documented in systemic autoimmunities with proven lymphoma links.[38]

B Cells in the Pancreatic Niche

Within islets and their draining nodes, these B cells double as antigen presenters and cytokine factories, fueling T-cell autoaggression while securing their own persistence via BAFF signaling and NF-κB activation. This built-in survival wiring directly parallels the antiapoptotic machinery of low-grade B-cell lymphomas. Viewed this way, T1D's B-cell pool sustains an active, though spatially boxed-in, clonal expansion that runs alongside the more visible insulitis.[39]

Clinical Evidence from Interventions

Therapeutic trials bring this B-cell role into sharp focus. In a rigorous placebo-controlled study, teplizumab-mediated B-cell depletion in early T1DM meaningfully slowed β-cell loss, proving that pruning these expanded clones delivers tangible metabolic gains. The effect's scale and staying power echo lymphoma biology: neutralizing a ruling clonal faction reshapes disease course, even short of full eradication.[40]

T-Cell Parallels and Interferon Drive

The story extends to T cells through interferon-α-fueled JAK–STAT activation, a cornerstone of T1DM peri-islet inflammation. Enduring STAT1 activity and type I interferon traces prop up T-cell programs strikingly similar to those in select T-cell lymphomas, hinting at a parallel, non-invasive clonal undercurrent in the T-cell realm.[41]

Epidemiologic Clues

People with T1D face a small but steady uptick in NHL risk versus the broader population. While outright cases stay rare, this pattern's consistency across studies points to chronic lymphocyte goading by antigen occasionally tipping into unchecked growth. T1D, then, likely harbors a quiet lymphoproliferative thread, one that seldom erupts into frank cancer but traces a unified biological gradient, not a sudden leap.[42]

Rheumatoid arthritis and the Dose–Response Link to Lymphomagenesis

RA delivers the most compelling population-level evidence that chronic autoimmunity and lymphoma development form a seamless biological spectrum. Long-term cohort analyses reveal a clear, stepwise relationship between accrued inflammatory load and lymphoma emergence. Individuals with unrelenting high disease activity face dramatically heightened risk, sometimes dozens of times above those in stable remission, who near general population levels, while moderate control proportionally tempers the threat. This dose-response pattern defies explanations of random malignant leaps, gaining clarity when viewed as dual facets of one antigen-fueled clonal dynamic.[43]

Synovium as Ectopic Lymphoid Hub

The joint's rheumatoid synovium morphs into a makeshift lymphoid organ, hosting structured aggregates with distinct T- and B-cell domains, follicular dendritic meshes, and high endothelial venule mimics. Local CXCL13 secretion draws and arranges B cells, recreating a miniature lymph node blueprint right within

the inflamed tissue. Here, autoreactive B cells expand clonally, rack up somatic mutations, and hone affinity amid constant antigen pressure.[44]

Shared Survival Machinery

Persistent NF-κB firing, BCR engagement, and BAFF-driven lifelines operate in this milieu, mirroring the anti-death scaffolding of marginal zone and other low-grade B-cell lymphomas. In typical RA, though, this growth engine stays tethered to antigen cues and joint-bound, falling short of the self-sustaining invasion that signals frank lymphoma.[45]

Remission as Proliferative Brake

Effective therapy does more than ease pain, it dials back this active lymphoproliferative engine. The lymphoma risk drop tied to lasting inflammation control underscores RA lymphoid surge as no mere bystander reaction, but a tangible step along the road to overt neoplastic growth.[46]

Psoriasis and Psoriatic arthritis: A T-Cell–Dominant Lymphoproliferative Continuum

Psoriasis and psoriatic arthritis reveal how a non-invasive lymphoproliferative dynamic can root primarily in the T-cell realm, with inflamed skin serving as a structured immune niche much like the ectopic germinal centers in other autoimmune contexts. Psoriatic lesions transcend simple cytokine storms; they form deliberate microenvironments primed for enduring antigen display, clonal T-cell buildup, and cytokine-fueled survival cues.

T-Cell Clonal Drive in Skin

Lesional skin accumulates Th1 and Th17 cells under relentless antigenic nudge. T-cell receptor (TCR) profiling shows oligoclonal bursts of skin-tropic T cells, pointing to targeted, chronic antigen shaping over haphazard recruitment. The IL-23/IL-17 network keeps this going by boosting Th17 differentiation, longevity, and potency. Strikingly, this pathway also props up malignant T cells in primary cutaneous T-cell lymphomas like mycosis fungoides, drawing a direct line from psoriatic flares to T-cell neoplastic growth.[47]

B Cells Play a Supporting Role

T cells steal the spotlight clinically, but B cells stir too. Elevated globulins, long-haul plasma cell growth, and NF-κB firing in keratinocytes plus infiltrates signal wider lymphoid momentum. This NF-κB-orchestrated transcription amps up cytokines and cell resilience, mimicking blueprints from both B- and T-cell lymphomas. Psoriasis thus likely harbors a two-pronged lymphoproliferative setup, T-cell forward with B-cell backup.[48]

Risk Patterns in the Data

Studies flag higher lymphoma odds, especially cutaneous T-cell types, in severe psoriasis cases. Absolute numbers stay low, yet the trend holds firm across groups, arguing for true biological overlap over chance. Progression to lymphoma here isn't inflammation flipping malignant switch; it's a pre-wired, antigen-tied clonal T-cell pool breaking free for unchecked expansion in perpetually irritated skin.[49]

Vitiligo: Autoreactive Cytotoxicity and the Paradox of Tumor Protection

Vitiligo pushes the model further, showing how enduring, antigen-targeted T-cell buildup can fuel both destructive autoimmunity and unexpected tumor defense. Defined clinically by white skin patches from melanocyte loss, vitiligo hinges immunologically on amassed $CD8^+$ killer T cells homing in on melanocyte markers. Both skin-resident and blood-borne $CD8^+$ T cells show narrowed T-cell receptor patterns, signaling focused, long-term antigen sculpting over generic inflammation.[50]

Molecular Survival Traits

At the gene level, these melanocyte-attacking $CD8^+$ T cells gear up for persistence, with boosted TCF7 (TCF-1) expression, anti-death factors like BCL-2, and shields against Fas-triggered cell suicide. Such wiring nurtures durable effector and skin-memory T cells. Tellingly, this endurance toolkit mirrors early cutaneous T-cell lymphomas, where constant antigen prodding and death resistance set the stage for unchecked growth. Vitiligo, then, nurtures a boxed-in, antigen-tied clonal T-cell surge, lethal to pigment cells yet held in check spatially.[51]

The Melanoma Paradox

Here's the twist: this same killer machinery sharpens defenses against melanoma. Studies routinely find lower melanoma rates in vitiligo patients, and treatment-induced vitiligo-like skin lightening flags better cancer responses. Shared-antigen $CD8^+$ T cells hit both healthy melanocytes and tumor cells, yielding antitumor side benefits from autoimmune crossfire.[52]

Lymphoma Risk in Context

Large-scale data hint at slightly higher NHL odds in widespread, long-term vitiligo, though cases stay

rare. This may trace to the toll of nonstop T-cell goosing and survival cues, which seldom, but sometimes, slip antigen's leash.[53]

Vitiligo captures a clonal T-cell engine driving targeted self-attack while bolstering cancer watch. Outcome, skin whitening or tumor kill, hinges less on the clone's wiring than its target menu.

Celiac disease: Antigen Dependence and the Reversibility of Clonal T-Cell Expansion

Celiac disease stands out as a strikingly clear example of how relentless antigenic pressure can nurture a clonal lymphoid buildup that stays biologically tethered—until it breaks free into autonomous proliferation. In active disease, gluten intake in genetically at-risk people, mostly those with HLA-DQ2 or HLA-DQ8, sparks an enduring immune attack on deamidated gliadin fragments displayed in the small bowel. The signature under the microscope: a surge of intraepithelial lymphocytes (IELs), mainly $CD8^+$ T cells, many sporting oligoclonal T-cell receptor patterns that scream antigen sculpting, not random inflammatory noise.[54]

Survival Wiring in the Gut Mucosa

These IELs carry activation tags and tissue anchors like CD103 and NKG2D, plus endurance tricks such as apoptosis shields and JAK1–STAT3 pathway ignition. That exact signaling backbone drives enteropathy-associated T-cell lymphoma (EATL), a rare but ferocious gut cancer that almost always sprouts from chronic celiac terrain. So active celiac hosts an antigen-bound, clonally pumped T-cell crew in its lining, molecular twins to EATL, just minus the self-fueling spark.[55]

The Power of Antigen Removal

What seals this beyond mere reaction? Its unwindability. A strict gluten-free diet yanks the trigger antigen, normalizing IEL numbers, dialing back clonal sway, and slashing lymphoma odds dramatically. This mirrors gastric MALT lymphoma fading after wiping out its bacterial antigen cue, hammering home that some lymphoid surges need constant antigenic fuel to persist.[56]

Risk Trajectory and Model Clarity

Population data peg untreated or stubborn celiac with 6- to 19-fold EATL hikes, measuring the lymphomagenic strain from nonstop T-cell revving. The arc, gliadin hit, sustained T-cell firing, clonal swell, EATL tipping point, offers a crystal model where pulling antigen halts the proliferative chain before it locks into irreversibility.[57]

Crohn's Disease: Microbial Triggers and Lymphoid Growth Imbalance

CD broadens the model by showing that chronic lymphoid buildup doesn't need classic autoantigens—ongoing reactions to gut microbes can spark a parallel, antigen-tied proliferative state. The deep-wall inflammation fosters organized MALT complete with follicles, germinal center mimics, and active B cells right in the intestinal layers. This setup echoes, at least partially, the tissue layout of extranodal marginal zone lymphomas in other persistently prodded sites.[58]

B Cells Shaped by Bacterial Cues

B cells pulled from inflamed gut linings carry somatic hypermutations and selection scars, proving steady clashes with bacterial bits over scattershot activation. A soup of TNF, IL-6, and BAFF nurtures their survival and maturation, while NF-κB hums nonstop in immune and gut cells alike. Clonality in CD runs milder than in Sjögren's or celiac hotspots, but structured lymphoid clusters plus constant microbial nudge place it firmly on the mucosal proliferation spectrum.[59]

Population Risk Patterns

Large-scale studies clock a 1.5- to 2.5-fold NHL uptick in inflammatory bowel disease, tied to flare intensity and immune-suppressing drugs. This tempered risk fits CD broader antigen targeting and looser clonal grip compared to sharper autoimmune profiles.[60]

Therapy's Double-Edged Warning

A stark lesson comes from thiopurine plus anti-TNF combos linked to hepatosplenic T-cell lymphoma—a rare, deadly $CD8^+$ T-cell cancer hitting young men hardest. It hints that meddling with immune balance can snap antigen tethers on bloated T-cell pools, unleashing runaway growth.[61]

Together, these signs frame CD treatments as modulators of a living, microbe-fueled lymphoid network, one normally kept in check, but vulnerable to tipping toward cancer under pressure.

Systemic Lupus Erythematosus: Circulating Lymphoid Proliferation Unleashed

SLE embodies the model's systemic incarnation, a diffuse lymphoproliferative state unbound by single-organ confines. Instead of localized lymphoid hubs, SLE unleashes autoreactive B cells across circulation and secondary lymphoid stations. Skyrocketing globulins, sprawling autoantibody profiles, and nonstop plasma blast churn signal relentless, systemic antigen goading.[62]

Enduring Antigen Shaping

Immunoglobulin gene sequencing from lupus B cells reveals somatic mutations and affinity honing, fingerprints of chronic antigen grooming, not haphazard firing. Surging serum BAFF hands these clones survival edge, rescuing them from natural purge. Nonstop NF-κB and JAK–STAT pulses lock in anti-death wiring, propping up bloated B-cell ranks in the bloodstream. Together, this forms a wandering, antigen-chained lymphoid engine, missing fixed turf, but packing full lymphomagenic toolkit.[63]

Shared Genetic Roots

SLE genomic wiring offers rock-solid proof of lymphoma kinship. GWAS pinpoints risk spots like BLK, PTPN22, TNFAIP3, and STAT4, key players in B-cell signaling, tolerance checks, and cytokine handling. Many overlap NHL variants, hinting at common DNA scaffolding steering both endless self-reactivity and outright cancer. Lymphoma here isn't a bolt-from-the-blue; it's the ramped-up endgame of a hardwired lymphoid script.[64]

Risk Amplifiers and Viral Partners

Epidemiologically, SLE packs a hefty NHL punch, especially diffuse large B-cell type. Epstein-Barr virus adds a twist: weakened viral curbs in SLE may seed growth nudges into an already swollen autoreactive B pool. SLE thus captures a fluid, blood-borne non-invasive proliferation where genes, antigen grind, and bugs collide on one oncogenic road.[65]

Multiple sclerosis: Chronic Antigenic Stimulation Within a Privileged Niche

MS showcases how enduring lymphoid goading can thrive mainly within the central nervous system (CNS), a site shielded anatomically and immunologically. Instead of lumped neoplastic growths, MS inflammation features long-term stimulated lymphocytes lingering in meninges, vessel cuffs, and cerebrospinal fluid. Myelin-targeting $CD4^+$ Th1 and Th17 cells stoke local flares, while clonally grown B cells and plasma cells drive intrathecal antibody production and oligoclonal bands, clear signs of boxed-in, antigen-fueled selection.[66]

B Cells Tuned by CNS Cues

Though myelin oligodendrocyte glycoprotein and aquaporin-4 (AQP4) antibodies tie more to kindred demyelinating ills, sustained B-cell firing in the CNS anchors MS pathology. Deep sequencing uncovers somatic mutations and affinity refinement in brain-bound B cells, matching germinal center action in meningeal clusters. This paints a picture of a steady-stimulated lymphoid pocket, propped by nonstop antigen nudges.[67]

JAK–STAT as Shared Wiring

Molecularly, JAK–STAT pathway convergence links MS immunity to lymphoid buildup. Downstream STAT1 and STAT3 kicks from IL-6, IL-23, and type I interferons back Th17 growth and B-cell endurance—circuits key to T-cell cancer origins too. The match doesn't equate autoimmunity with tumor, but spotlights common controls for lymphocyte staying power, spread, and death resistance.[68]

Natalizumab's Balancing Act

Natalizumab's track record illuminates MS immune poise. Blocking α4-integrin lymphocyte entry into the CNS quiets flares but jostles surveillance. Rarely, this shift sparks CNS lymphoid ills, often via Epstein-Barr virus resurgence. It hints that normally antigen-tied, controlled lymphocyte crews can bolt toward self-fueling growth when oversight falters.[69]

Risk in the Population View

Studies note a slight NHL bump in MS—roughly 1.4–2.0-fold over general rates. For most, though, antigen reliance and solid regulation keep this CNS-trapped lymphoid hum in safe, non-cancerous bounds lifelong.[70]

CONCLUSION

Autoimmune diseases and indolent lymphomas converge along a shared biological continuum defined by chronic antigenic stimulation, clonal lymphocyte expansion, and activation of conserved survival pathways. Rather than discrete entities, these conditions reflect graded expressions of a common lymphoid growth program. Recognizing systemic autoimmunity as a non-infiltrative lymphoid neoplastic state reframes surveillance, therapeutic strategy, and malignant risk interception.

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REVIEW *REVISÃO*

OZEMPIC BABIES: MECHANISMS AND IMPACTS OF SEMAGLUTIDE THERAPY

"OZEMPIC BABIES": MECANISMOS E IMPACTOS DA TERAPIA COM SEMAGLUTIDA

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ABSTRACT

Introduction: Celebrities and social media influencers have attributed their weight loss to the use of Ozempic® (semaglutide), a medication intended to control diabetes mellitus 2 (DM2). Some women have reported increased fertility and unplanned pregnancies after using Ozempic®, giving rise to the term «Ozempic Babies." In 2015, a study observed increase of luteinizing hormone (LH) peaks compatible with the preovulatory phase in rats, thus establishing a possible link between semaglutide and fertility. **OBJECTIVES:** To review the use of Ozempic® for weight loss and probable mechanisms of increased fertility. **Methodologies:** Systematic review, from May to July 2024, using databases: PubMed, Nature, DynaMed. RESULTS: Glucagon type 1 peptide receptor analogs (GLP-1ra), used in the treatment of T2DM, help reduce body mass by modulating insulin action. Body weight reduction in women with polycystic ovary syndrome has always been associated with fertility, generating a decrease in both insulin resistance and hyperandrogenism. Based on studies suggesting altered dynamics in GLP-1ra secretion in hyperandrogenism, researchers have demonstrated that in animals, GLP-1ra can promote pulsatility of LH secretion and restore ovulation. It is important to emphasize that there are no solid safety studies on GLP-1ra in pregnancy, and the medication should be discontinued during prenatal planning. **Conclusion:** GLP-1ra appears to affect female fertility by promoting preovulatory physiological peaks of LH. Reports of unplanned pregnancies indicate the need for caution for women of childbearing age.

Keywords: GLP-1ra; Fertility; Pregnancy.

RESUMO

introdução: Celebridades e influenciadores de mídias sociais têm atribuído sua perda de peso ao uso de Ozempic® (semaglutida), uma medicação destinada ao controle do *Diabetes Mellitus tipo 2* (DM2). Algumas mulheres relataram aumento da fertilidade e gestações não planejadas após o uso de Ozempic®, dando origem ao termo «Ozempic Babies". Em 2015, um estudo observou picos aumentados de hormônio luteinizante (LH) compatíveis com a fase pré-ovulatória em ratas, estabelecendo assim uma possível ligação entre a semaglutida e a fertilidade. **Objetivos:** Revisar o uso de Ozempic® para perda de peso e os prováveis mecanismos de aumento da fertilidade. **Metodologias:** Revisão sistemática, realizada de maio a julho de 2024, utilizando as bases de dados: PubMed, Nature, DynaMed. **Resultados:** Análogos do receptor do peptídeo 1 semelhante ao glucagon (GLP-1ar), utilizados no tratamento do DM2, auxiliam na redução da massa corporal pela modulação da ação da in-

sulina. A redução do peso corporal em mulheres com síndrome dos ovários policísticos sempre foi associada à fertilidade, gerando diminuição tanto da resistência à insulina quanto do hiperandrogenismo. Com base em estudos que sugerem dinâmica alterada na secreção de GLP-1ar no hiperandrogenismo, pesquisadores demonstraram que em animais, o GLP-1ar pode promover a pulsatilidade da secreção de LH e restaurar a ovulação. É importante enfatizar que não existem estudos de segurança sólidos sobre o GLP-1ar na gestação, e a medicação deve ser descontinuada durante o planejamento pré-natal. **Conclusão:** O GLP-1ar parece afetar a fertilidade feminina promovendo picos fisiológicos pré-ovulatórios de LH. Relatos de gestações não planejadas indicam a necessidade de cautela para mulheres em idade fértil.
Palavras-chave: GLP-1ar; Fertilidade; Gravidez.

INTRODUCTION

Celebrities and digital media influencers have reported and attributed their weight loss to the use of Ozempic® (semaglutide), even though it is not intended for this purpose but rather for controlling Diabetes Mellitus 2 (DM2).[1,2]

These statements, although not officially sponsored, helped boost the popularity of the drug among women seeking rapid weight loss, in some cases even without becoming obese.[1]

For many women, self-esteem is closely tied to weight loss, a fact that is often exacerbated by societal beauty standards that favor thin bodies. This can lead to the pursuit of quick methods to achieve the 'ideal' body seen on models, television actresses, and magazine covers.[3]

Thus, when celebrities and influencers obtain results with Ozempic® and share them on social media, this mainly induces the public to seek medication to lose weight and improve their self-esteem, as they are the main target of this social pressure.[1,2,3]

Weight loss positively impacts women's self-esteem, reflected in several aspects of their lives, including sexual relationships and fertility. Many women report feeling more confident and comfortable after losing weight, which can improve their quality of life and sexual well-being. Within this context, this systematic review aims to review the widespread use of Ozempic® and its influence on increasing female fertility, addressing physiological and endocrine changes in women. [1,3]

However, some women have reported increased fertility after using Ozempic®, becoming pregnant with unplanned babies[4], known as "Ozempic Babies."[1] In 2015, a study was published in which they found that dosing female rats with GLP-1ra stimulated the production of luteinizing hormone (LH)[4]. Thus, they report that GLP-1ra receptor analogs promote fertility because they can increase the preovulatory surge of luteinizing hormone (LH)[4].

Within this context, this systematic review aims to review the widespread use of Ozempic® and its influence on increasing female fertility, addressing physiological and endocrine changes in women.

METHODOLOGIES

The current literature review was carried out from May to August 2024. The material for reading and analysis was selected from research on virtual platforms, such as PubMed, Nature, and Scielo. A selective search was carried out for descriptors in Portuguese, English, and Spanish related to the topic, using the following keywords: "obesity," "fertility," "glucagon-like peptide one receptor agonist," and polycystic ovary, using articles published from 2020 onwards. A total of 25 references were found, of which seven were excluded because they were not wholly related to the article's topic. The inclusion and exclusion criteria of the articles were based on the sources where they were published, the relevance of the subject addressed, and the correspondence with the article's topic. These articles were read by title and abstract, followed by the entire reading of those with more significant relation to the topic, finalizing 18 articles. The bibliographic work "Treatise on Medical Physiology Guyton and Hall" was also used. Ethical aspects were strictly respected.

RESULTS AND DISCUSSION

Introducing the hormone and its receptor:

Ozempic® is an injectable medication containing the active ingredient semaglutide,[11] a glucagon-like

peptide-1 receptor agonist (GLP-1ra) for treating adults with type 2 diabetes mellitus.[5,6,8] GLP1-ra is especially indicated in cases where glycemic control in individuals who are overweight/obese are not satisfied with diet and exercise alone, that metformin is contraindicated or not well tolerated, and when the combination of GLP1-ar with other antidiabetic drugs is necessary.[5,6]

Glucagon-like peptide-1 (GLP-1) is a hormone produced when intestinal epithelial endocrine L cells process proglucagon after a meal.[7] GLP-1 receptors are expressed in various tissues, including the intestine, heart, kidney, brain, immune cells, pancreatic islets, and ovaries.[7] Each tissue responds to GLP-1ra with distinct pharmacological actions and encompasses various metabolic and physiological processes.[7]

Physiology of the menstrual cycle:

By activating the GLP-1 receptor, semaglutide promotes the proliferation of pancreatic beta cells, suppressing appetite, regulating lipid metabolism, and improving insulin resistance.[8] The first GLP-1ra was launched in Brazil in 2007 for subcutaneous use twice daily (Byetta®).

Subsequently, the once-daily dose of liraglutide (Victoza® and Saxenda®) and the weekly dose of dulaglutide (Trulicity®) popularized the concept of innovative treatment of T2DM associated with weight loss.[8]

Balanced eating plans, a stable family environment, and regular physical activity all contribute to a favorable balance for women's health, promoting ovulatory menstrual cycles and increasing the chance of pregnancy[9]. Therefore, the physiological ideal for a future pregnancy would be to have menstrual cycles with regular intervals, between 21 and 35 days, and presumed ovulation 14 days before vaginal bleeding, signaling a mature system ready for conception.[10] It is also essential that the reproductive organs, such as the uterus, fallopian tubes, and ovaries, are healthy and free of diseases that may affect fertility.[10]

Hormone levels, including estrogen and progesterone, must be balanced to regulate the menstrual cycle, ovulation, and endometrial receptivity.[10] In general health, the ideal body mass index (BMI) would be between 18.5 and 24.9 kg/m², which is associated with tremendous success in conceiving and maintaining pregnancy during the period necessary for complete fetal development.[11] Qualitatively and quantitatively, adequate nutrition is described as isocaloric, normoproteic, and balanced in unsaturated fats and slow absorption carbohydrates. If there are special needs, supplementation with folic acid, iron, calcium, and vitamin D will be necessary.[12]

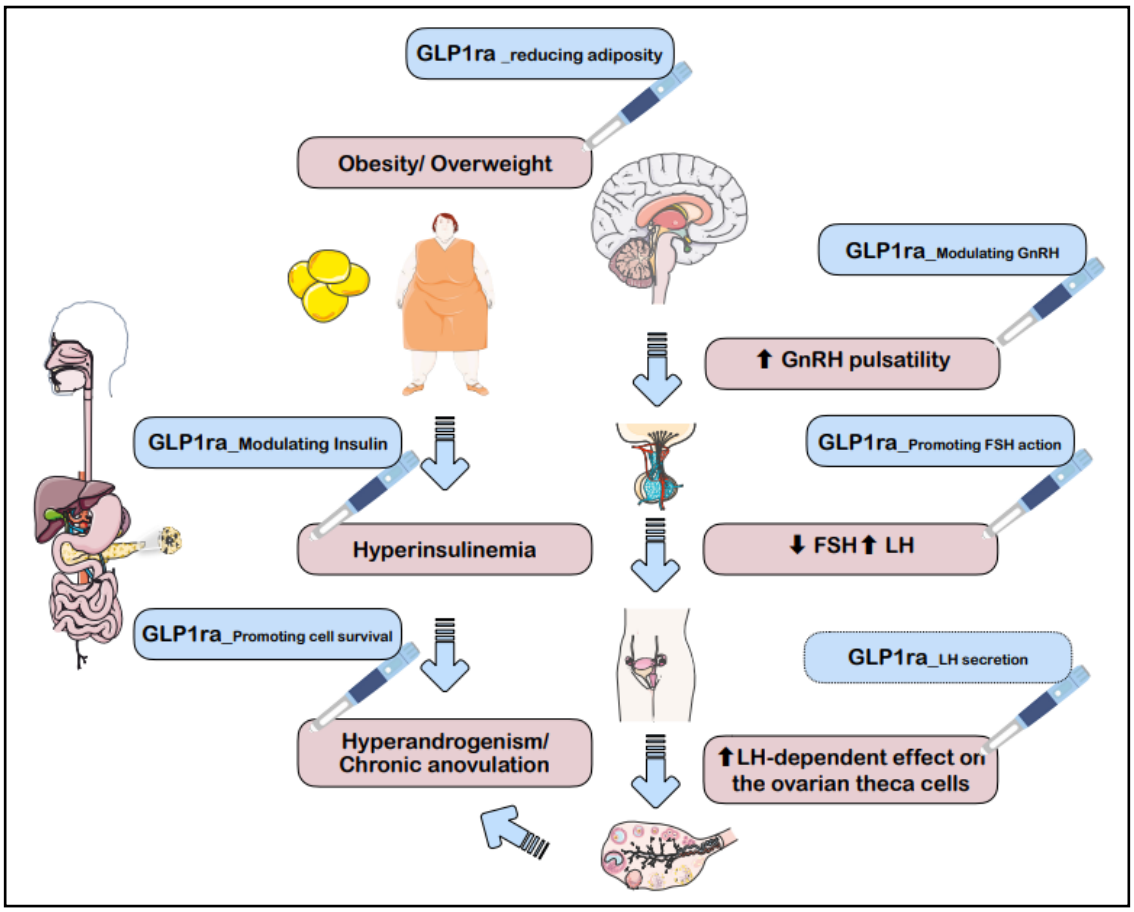
On the other hand, overweight or obese women[13] may experience delayed conception, as they have reduced fertility that is often associated with hormonal disorders, such as hyperandrogenism, insulin resistance, and polycystic ovary syndrome (PCOS), which interfere with ovulation.[14] The common mechanism described in chronic anovulation would be due to the tone of luteinizing hormone (LH), requiring additional stimuli from follicle-stimulating hormone (FSH)[4] for the adequate development of ovarian follicles. In this way, many follicles are stimulated but do not reach maturation for the correct follicle rupture and production of a corpus luteum capable of maintaining progesterone levels compatible with the implantation of the future egg.[14,15]

In addition to LH tone, it is described that the state of insulin resistance promotes the activation of theca cells, worsening hyperandrogenism and also, by reducing the hepatic production of SHBG (sex hormone binding globulin), increases the proportion of free androgens in the blood, worsening the anovulation cycle and polycystic ovary syndrome.[15,16] Because of this, overweight and obesity in the female population can translate into a higher frequency of anovulatory cycles, oligomenorrhea, and hirsutism.[16]

Although it has long been known, and based on peer-reviewed scientific studies, that weight loss (fat mass) is related to a higher success rate in the intention to conceive. Thus, as with the return of menstrual cycles in women with polycystic ovary syndrome, the novelty of Ozempic® in increasing fertility is due, in particular, to social media and unplanned pregnancies, giving rise to the nickname «Ozempic babies" [1]. It has attracted much attention, both from the medical community and the lay press, that some women using semaglutide reported getting pregnant even while taking the birth control pill. [4] And other women who were previously diagnosed as infertile became pregnant after using semaglutide.[4,8]

Currently, attempts are being made to explain the phenomenon that GLP1-ra, by promoting weight loss and reducing lipotoxicity, restores cellular function in ovarian cells[8]. It is also known that GLP-1 acts about fertility independently of weight loss since it can increase the production of the LH hormone, which triggers ovulation[4]. In users of oral contraceptives, it is worth remembering that GLP-1ra, by slowing gastrointestinal motility, can reduce the absorption of medications, making them less effective[4]. Among the GLP-1ar, semaglutide showed a 66% reduction in the maximum concentration of contraceptives in the blood and is therefore considered a pregnancy inducer among women who use this drug[4].

Figure 1. Disruption of the HPG axis in PCOS and possible beneficial effects of GLP-1 RA. GLP-1/RA, glucagon-like peptide 1 receptor agent; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin.



Problems with semaglutide and analogs in general:

Semaglutide offers significant benefits for reproductive health by addressing multiple aspects that affect fertility, but little is known about the safety and long-term effects of its actions on embryonic development.[17] Thus, what facilitates conception in women with difficulty getting pregnant may contribute to the arrival of "Ozempic babies" with characteristics and challenges that have not yet been studied.[1,8]

Studies on the effects and safety of long-acting incretins in pregnancy in animals have revealed that in rats, rabbits, and mice, GLP-1 receptor agonists, in addition to reducing fetal weight, show delayed ossification and a more significant occurrence of skeletal anatomical variants.[18]

Comparatively, the scarce case reports of pregnant women undergoing treatment with semaglutide indicated that discontinuation of the medication upon detecting pregnancy or maintaining use until the 5th week of gestation was not associated with a significant increase in the risk of severe congenital disabilities.[19,20] Using semaglutide reported getting pregnant even while taking the birth control pill. And other women who were previously diagnosed as infertile became pregnant after using semaglutide. [20]

Thus, due to the scarcity of data on the use of semaglutide and gestational outcomes and even during lactation, the manufacturer recommends stopping treatment two months before the planned conception.[4]

CONCLUSION

The rampant use of Ozempic® and its influence on female fertility reveals that although the drug was developed to treat type 2 diabetes, its side effects, especially weight loss, have significantly impacted women›s reproductive health. Physiological changes and endocrine changes resulting from using semaglutide, such as stimulating LH hormone production and reducing lipotoxicity, contribute to restoring ovarian

function and improving fertility. Reports of unplanned pregnancies, known as "Ozempic babies", highlight the need for caution and monitoring when prescribing the drug to women of reproductive age. Studies indicate that semaglutide may promote ovulation and interfere with the effectiveness of oral contraceptives, increasing the risk of pregnancy. Although preliminary results are promising, more research is needed to understand the long-term effects on human fertility. This review highlights the experts' recommendation that women taking the drug consult their doctors about appropriate contraceptive methods and consider stopping treatment two months before planned conception to minimize possible risks to reproductive health and fetal development. Therefore, it is clear that in addition to its role in controlling diabetes and aiding in weight loss, Ozempic® has a potential impact on female fertility that needs to be carefully evaluated and monitored.

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CASE REPORT *RELATO DE CASO*

TALL STATURE AS A CLUE TO AN UNDERLYNG GENETIC CONDITION

ALTA ESTATURA COMO PISTA PARA UM DISTÚRBIO GENÉTICO

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ABSTRACT

Klinefelter syndrome is the most common sex chromosome aneuploidy in males and an important cause of primary hypogonadism and male infertility. It is characterized by the presence of an additional X chromosome, resulting in the 47,XXY karyotype, and may present with variable clinical manifestations, including tall stature, gynecomastia, small testes, and neurocognitive alterations. We report the case of a 16-year-old adolescent referred for evaluation of excessive growth and gynecomastia present since childhood. The patient had a history of childhood depression, episodes of aggressiveness, poor academic performance, and recurrent knee pain, and was receiving fluoxetine. Physical examination revealed a eunuchoid habitus, height of 196 cm (above the 97th percentile), weight of 73 kg, and body mass index of 23.15 kg/m². Genital examination showed Tanner stage V pubic hair and penile length, but small testicular volume (4–5 mL) and bilateral gynecomastia. Hormonal evaluation demonstrated elevated levels of FSH and LH associated with reduced testosterone levels, consistent with primary gonadal failure. Karyotype analysis confirmed Klinefelter syndrome (47, XXY), and bone age was estimated between 14 and 15 years.Tall stature in these patients may be related, among other factors, to the overexpression of SHOX gene, located in the pseudoautosomal region of the sex chromosomes. This case highlights the importance of genetic investigation in patients presenting with tall stature associated with signs of hypogonadism.

Keywords: Klinefelter Syndrome; Hypogonadism; Gynecomastia.

RESUMO

A síndrome de Klinefelter é a aneuploidia cromossômica sexual mais comum em indivíduos do sexo masculino e uma importante causa de hipogonadismo primário e infertilidade masculina. Caracteriza-se pela presença de um cromossomo X adicional, resultando no cariótipo 47,XXY, e pode apresentar manifestações clínicas variadas, como estatura elevada, ginecomastia, testículos pequenos e alterações neurocognitivas. Relata-se o caso de um adolescente de 16 anos encaminhado para investigação de crescimento excessivo e ginecomastia presente desde a infância. O paciente apresentava história de depressão infantil, episódios de agressividade, baixo desempenho escolar e dor recorrente em joelhos e estava em uso de fluoxetina. Ao exame físico observou-se biotipo eunucóide, estatura de 196 cm (acima do percentil 97), peso de 73 kg e índice de massa corporal de 23,15 kg/m². O exame genital revelou estágio V de Tanner para pelos pubianos e comprimento peniano, porém com testículos de pequeno volume (4–5 mL) e ginecomastia bilateral. A avaliação hormonal demonstrou níveis elevados de FSH e LH, associados a níveis reduzidos de testosterona, compatíveis com falência gonadal primária. O cariótipo confirmou síndrome de Klinefelter (47,XXY), e a idade óssea foi estimada entre

14 e 15 anos. A estatura elevada nesses pacientes pode estar relacionada, entre outros fatores, à superexpressão do gene SHOX, localizado na região pseudoautossômica dos cromossomos sexuais. Este caso ressalta a importância da investigação genética em pacientes com estatura elevada associada a sinais de hipogonadismo.

Descritores: Síndrome de Klinefelter; Hipogonadismo; Ginecomastia.

INTRODUÇÃO

Klinefelter syndrome is a genetic condition characterized by the presence of one or more additional X chromosomes in individuals with a male phenotype, with the 47, XXY karyotype accounting for approximately 80–90% of cases[1,2]. The estimated prevalence ranges from 1:500 to 1:1000 male births, corresponding to approximately 0.1–0.2% of the male population[3]. This chromosomal abnormality usually results from meiotic nondisjunction during maternal or paternal gametogenesis[4].

From a pathophysiological perspective, the presence of the extra chromosome leads to progressive testicular alterations, including fibrosis and hyalinization of the seminiferous tubules, as well as dysfunction of Leydig cells. Consequently, reduced spermatogenesis and decreased testosterone production occur, leading to hypergonadotropic hypogonadism, infertility, and characteristic phenotypic features[3,4]. Increased stature in these individuals is partly associated with overexpression of the SHOX gene, located in the pseudoautosomal region of the sex chromosomes, which contributes to longitudinal limb growth. Despite being the most common sex chromosome aneuploidy, a significant proportion of cases remain undiagnosed until adulthood due to the wide phenotypic variability[5,6].

CASE REPORT

A 16-year-old adolescent was referred to the endocrinology service for evaluation of excessive growth and gynecomastia present since childhood. The patient had a history of childhood depression, aggressiveness, poor school performance, and recurrent knee pain and was taking fluoxetine.

Physical examination revealed a eunuchoid habitus, height of 196 cm (above the 97th percentile), weight of 73 kg, and body mass index of 23.15 kg/m². Genital examination demonstrated Tanner stage V pubic hair and penile length, but small testes with a volume 4–5 mL and bilateral gynecomastia with periareolar glandular tissue. Hormonal investigation showed elevated FSH (40.25 IU/L) and LH (11.66 IU/L) levels associated with reduced testosterone (273 ng/dL), findings compatible with primary gonadal failure. IGF-1 levels were within the normal range for age. G-band karyotype analysis confirmed Klinefelter syndrome (47,XXY). Hand and wrist radiography demonstrated bone a bone age between 14 and 15 years.

Tall stature in these patients may be related, among other factors, to overexpression of the SHOX gene, located in the pseudoautosomal region of the sex chromosomes.Early recognition of this condition allows appropriate management of endocrine, reproductive, and psychosocial complications.

Figure 1. Bilateral gynecomastia.

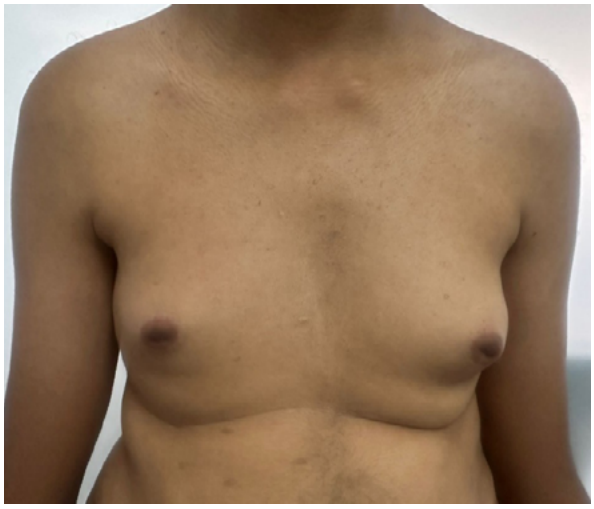
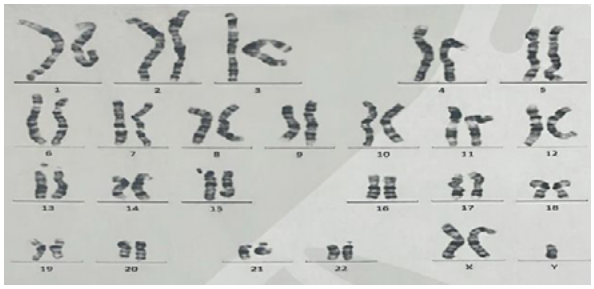


Figure 2. Karyotype of Klinefelter syndrome showing an extra X chromosome (47,XXY), differing from the typical male karyotype (46,XY). Blue circle highlighting the two X chromosomes.



DISCUSSION

Klinefelter syndrome has an estimated prevalence between 0.1% and 0.25% of the male population and is considered the most common genetic cause of primary hypogonadism and male infertility[1,2,7]. In the present case, investigation was prompted by the presence of stature significantly above the expected familial target height. The discrepancy between observed height and genetic potential raised suspicion of a pathological condition.Tall stature is a relatively common manifestation of Klinefelter syndrome and may represent a diagnostic challenge. It is defined as height greater than two standard deviations above the mean for age and sex[5]. Differential diagnosis includes normal variants such as familial tall stature, as well as several endocrine and genetic conditions. Endocrine causes include hyperthyroidism, pituitary gigantism due to growth hormone excess, and different forms of hypogonadism[4,6]. Other genetic syndromes associated with tall stature include Marfan syndrome, Sotos syndrome, Weaver syndrome, and homocystinuria.

In the present case, the association of tall stature, eunuchoid habitus, gynecomastia, and small testes raised suspicion of hypergonadotropic hypogonadism, later confirmed by elevated gonadotropin levels and the 47,XXY karyotype. Eunuchoid body proportions occur due to delayed epiphyseal closure associated with relative testosterone deficiency during pubertal development[5,6]. Beyond the hormonal component, tall stature in these patients is also related to overexpression of the SHOX gene. Individuals with the 47,XXY karyotype possess three copies of this gene — two on the X chromosome and one on the Y chromosome[6]. Because this gene is located in the pseudoautosomal region and escapes X-chromosome inactivation, all copies remain active, resulting in increased gene expression and enhanced longitudinal bone growth[2,7]. This phenomenon is known as the gene dosage effect, in which an increased number of gene copies leads to greater production of the corresponding protein and, consequently, specific phenotypic alterations[8]. Another relevant aspect in the evaluation was the exclusion of pituitary gigantism, since IGF-1 levels were within the normal range, making growth hormone excess unlikely. Gynecomastia occurs in up to 80–90% of patients with Klinefelter syndrome and mainly results from an increased estradiol-to-testosterone ratio[5,9]. This condition may have significant psychosocial impact and, in some cases, may require therapeutic intervention, including surgical treatment.

CONCLUSION

This case highlights the importance of careful clinical evaluation in the investigation of tall stature, particularly when there is a significant discrepancy with the patient's familial genetic potential. Recognition of clinical features such as eunuchoid habitus, gynecomastia, and reduced testicular volume should raise suspicion for Klinefelter syndrome and prompt cytogenetic investigation. Early diagnosis allows appropriate follow-up and management of clinical manifestations, as well as reproductive and psychosocial counseling.

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CASE REPORT *RELATO DE CASO*

CUSHING'S DISEASE CHALLENGES IN DIAGNOSIS: WHEN THE CHRONOLOGY OF LABORATORY TESTS NEEDS TO BE FOLLOWED! A CASE REPORT AND MINI REVIEW

DOENÇA DE CUSHING ARMADILHAS NO DIAGNÓSTICO: QUANDO A CRONOLOGIA DOS TESTES LABORATORIAIS NECESSITA SER SEGUIDA! UM RELATO DE CASO E MINI REVISÃO

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ABSTRACT

Cushing's disease (CD) is the leading cause of endogenous Cushing's syndrome (CS), resulting from excessive adrenocorticotropic hormone (ACTH) secretion by pituitary adenomas, leading to chronic hypercortisolism and its multiple systemic repercussions. Despite being well defined from a pathophysiological standpoint, its diagnosis remains challenging due to the insidious clinical presentation and overlap with prevalent conditions such as obesity, type 2 diabetes mellitus, and metabolic syndrome. This study aims to highlight the diagnostic pitfalls of CD, emphasizing the importance of proper sequencing and interpretation of laboratory tests. We report the case of a 53-year-old female patient with long-standing type 2 diabetes mellitus and multiple microvascular and macrovascular complications, associated with persistently inadequate glycemic control. On physical examination, she presented classic signs of hypercortisolism, including central obesity, moon facies, abdominal striae, and a dorsocervical fat pad. Initial investigation excluded an exogenous cause. Laboratory evaluation confirmed hypercortisolism, evidenced by elevated serum cortisol, late-night salivary cortisol, and urinary free cortisol levels, along with failure to suppress on the dexamethasone suppression test. Elevated ACTH levels characterized an ACTH-dependent condition, consistent with CD. This case illustrates the importance of a structured diagnostic approach based on biochemical confirmation of hypercortisolism prior to imaging studies, thereby avoiding misinterpretation due to incidentalomas. It also highlights the need to consider the limitations of laboratory tests, particularly in patients with comorbidities such as chronic kidney disease. In conclusion, CD should be suspected in patients with difficult-to- control metabolic syndrome and suggestive clinical features. Early diagnosis, based on careful clinical evaluation and an appropriate sequence of tests, is essential to reduce morbidity, guide appropriate treatment, and improve patient prognosis and quality of life.

Keywords: Cushing disease; Dexamethasone suppression test; Late-night salivary cortisol; Urinary free cortisol.

RESUMO

Resumo: A doença de Cushing (DC) é a principal causa de síndrome de Cushing (SC) endógena, resultante da secreção excessiva de ACTH por adenomas hipofisários, levando ao hipercortisolismo crônico e suas múltiplas reper-

cussões sistêmicas. Apesar de bem definida fisiopatologicamente, seu diagnóstico permanece desafiador devido à apresentação clínica insidiosa e à sobreposição com condições prevalentes, como obesidade, diabetes mellitus tipo 2 e síndrome metabólica. Este estudo tem como objetivo destacar as armadilhas diagnósticas da DC, enfatizando a importância da sequência e da interpretação adequada dos testes laboratoriais. Relata-se o caso de uma paciente de 53 anos com diabetes mellitus tipo 2 de longa data e múltiplas complicações micro e macrovasculares, associadas a controle glicêmico persistentemente inadequado. Ao exame físico, apresentava sinais clássicos de hipercortisolismo, como obesidade central, fácies em lua cheia, estrias abdominais e giba dorsocervical. A investigação inicial excluiu a causa exógena. A avaliação laboratorial confirmou hipercortisolismo, evidenciado por níveis elevados de cortisol sérico, salivar noturno e urinário, além de ausência de supressão no teste com dexametasona. A dosagem de ACTH elevada caracterizou um quadro ACTH- dependente, compatível com DC. O caso ilustra a importância da abordagem diagnóstica estruturada, baseada na confirmação bioquímica do hipercortisolismo antes da realização de exames de imagem, evitando interpretações equivocadas decorrentes de incidentalomas. Destaca-se também a necessidade de considerar limitações dos testes laboratoriais, especialmente em pacientes com comorbidades como doença renal crônica. Conclui-se que a DC deve ser suspeitada em pacientes com síndrome metabólica de difícil controle e manifestações clínicas sugestivas. O diagnóstico precoce, baseado em avaliação clínica criteriosa e sequência adequada de testes, é fundamental para reduzir a morbidade, orientar o tratamento adequado e melhorar o prognóstico e a qualidade de vida dos pacientes.

Descritores: Doença de Cushing; Teste supressão da dexametasona; Cortisol salivar à meia noite; Cortisol livre urinário.

INTRODUCTION

Cushing's disease (CD) is the most common cause of endogenous Cushing's syndrome (CS) and results from an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma. This condition leads to chronic hypercortisolism through persistent stimulation of the adrenal cortex, culminating in increased cortisol production and systemic metabolic dysregulation[1].

From a pathophysiological standpoint, the disease arises from dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, in which autonomous ACTH secretion by a corticotroph adenoma promotes continuous adrenal stimulation and excessive cortisol production, independent of physiological negative feedback mechanisms. As a consequence, the circadian rhythm of cortisol secretion is disrupted, with persistently elevated levels throughout the day — one of the most characteristic findings of the disease[2].

Despite being well established conceptually, CD remains frequently underdiagnosed or late-diagnosed. This is largely attributable to the insidious nature of its clinical manifestations, which often overlap with highly prevalent conditions such as obesity, metabolic syndrome, diabetes mellitus, depression, and alcoholism. Furthermore, pseudo-Cushing states, the use of interfering medications, and cyclic forms of the disease represent important sources of diagnostic error[3].

In this context, the diagnosis of CD requires a systematic approach based on laboratory tests that assess different aspects of cortisol physiology, including circadian rhythm, overall production, and the integrity of HPA axis feedback. However, beyond test selection, correct interpretation and, most importantly, adherence to the appropriate chronological sequence in which they are performed are critical determinants of diagnostic accuracy[1,3].

Given these limitations, initiating the investigation with imaging studies may lead to diagnostic misinterpretation, particularly in view of the high prevalence of pituitary and adrenal incidentalomas in the general population. Therefore, this case report aims to highlight the main diagnostic pitfalls in Cushing's disease, emphasizing proper test sequencing and the chronological interpretation of laboratory findings as essential elements for accurate diagnosis and appropriate clinical management.

CASE REPORT

A 53-year-old female patient with a diagnosis of type 2 diabetes mellitus (T2DM), first established at the age of 25 during pregnancy, has been on insulin therapy since the ageof43.Shepresentsmultiplediabetes-relatedtargetorgancomplications,including diabeticretinopathy,heartfailurewithpreservedejectionfraction(HFpEF;EF:54.9%), chronickidneydisease(CKD) stageG3bA3,anddiabeticneuropathy.Herpastmedical history is also notable for class II obesity (BMI: 35.1 kg/m²), hypothyroidism, systemic arterial hypertension, dyslipidemia, peripheral arterial disease, and a prior episode of deep veinthrombosis.

ThepatientwasreferredbytheCardiologyDepartmenttotheEndocrinologyoutpatient clinic at Hospital Universitário Evangélico Mackenzie for optimization of the metabolic management of T2DM, given the multiplicity of complications. On physical examination, a phenotype strongly suggestive of hypercortisolism was identified, characterized by central obesity, abdominal striae, moon facies, and dorsocervical fat accumulation (buffalo hump). Based on these findings, an investigation for Cushing's syndrome was initiated. Exogenous etiology was promptly excluded, as the patient reported no prior glucocorticoiduse.

Initial laboratory evaluation revealed chronically refractory glycemic control, with glycated hemoglobin (HbA1c) values ranging from 11.3% to 12.9%. Additionally, a progressive decline in renal function was documented: the estimated glomerular filtration rate (eGFR) was 44.86 mL/min/1.73m², accompanied by a marked increase in the urinary albumin-to-creatinine ratio (UACR), which progressed from 710 mg/g to 5,972 mg/g, and 24-hour proteinuria of 5,099 mg, consistent with nephrotic-range proteinuria.

Evaluation of the hypothalamic–pituitary–adrenal (HPA) axis confirmed hypercortisolism, with the following findings: morning basal serum cortisol of30.16 µg/dL, morning salivary cortisol of 3.250 µg/dL, post-dexamethasone suppression serum cortisol of 21.12 µg/dL, midnight serum cortisol of 28.87 µg/dL, and 24-hour urinary free cortisol of 545 µg/24h. Subsequent measurement of adrenocorticotropic hormone (ACTH) revealed an elevated level of 126 pg/mL, indicating an ACTH- dependent etiology.

Based on clinical and laboratory correlation, a diagnosis of Cushing's disease was established. For etiological characterization and assessment of complications secondary to chronic hypercortisolism, pituitary magnetic resonance imaging (MRI) and bone densitometry were requested, respectively.

Figure 1. Moon facies associated with periorbital edema, facial plethora, and telangiectasias, clinical findings suggestive of hypercortisolism.

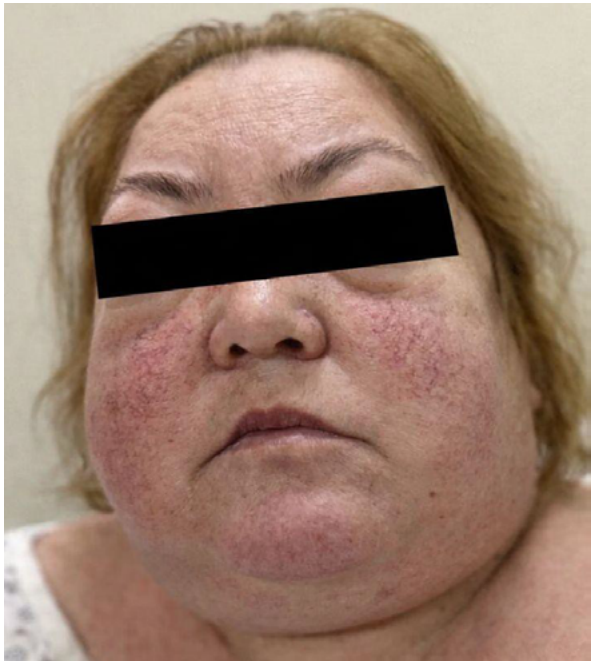


Table 1. Laboratory biochemical analysis of the patient.

Laboratory Test (Unit of Measurement)	Current Result	Previus Results	Reference Range
Glomerular Filtration Rate (mL/min/1,73 m²)	44	36 → 48	≥ 90
Albuminuria (mg/g)	5099	710 → 5972	< 30
Glycated Hemoglobin (%)	12,40	11,3 → 12,9	< 7 in patients with DM
Post-Dexamethasone Cortisol (µg/dL)	21,12	—	≤1.8
Morning Salivary Cortisol (µg/dL)	3,250	—	< 0,736
24h -Hour Urinary Free Cortisol (µg/24h)	545	509	58,0 to 403,0
Serum Cortisol (µg/dL)	28,87 (collected at 00h)	30,16 (collected at 09h)	6,70 to 22,60 (morning collection)
ACTH (pg/mL)	126	—	< 46

DISCUSSION

Clinical, Laboratory, and Imaging Diagnosis

Clinical Presentation

Patients with Cushing's disease present characteristic clinical manifestations resulting from prolonged exposure to excess cortisol. These include central obesity, moon facies, skin thinning, proximal muscle weakness, arterial hypertension, glucose intolerance or diabetes mellitus and neuropsychiatric symptoms. These findings may be accompanied by more specific signs — including wide violaceous striae, early-onset osteoporosis, hypogonadism, and menstrual irregularities — which further support the clinical diagnosis[2].

Despite these relatively distinctive features, diagnosis is frequently delayed, as many manifestations develop insidiously and overlap with more common conditions, such as obesity and metabolic syndrome[2,3].

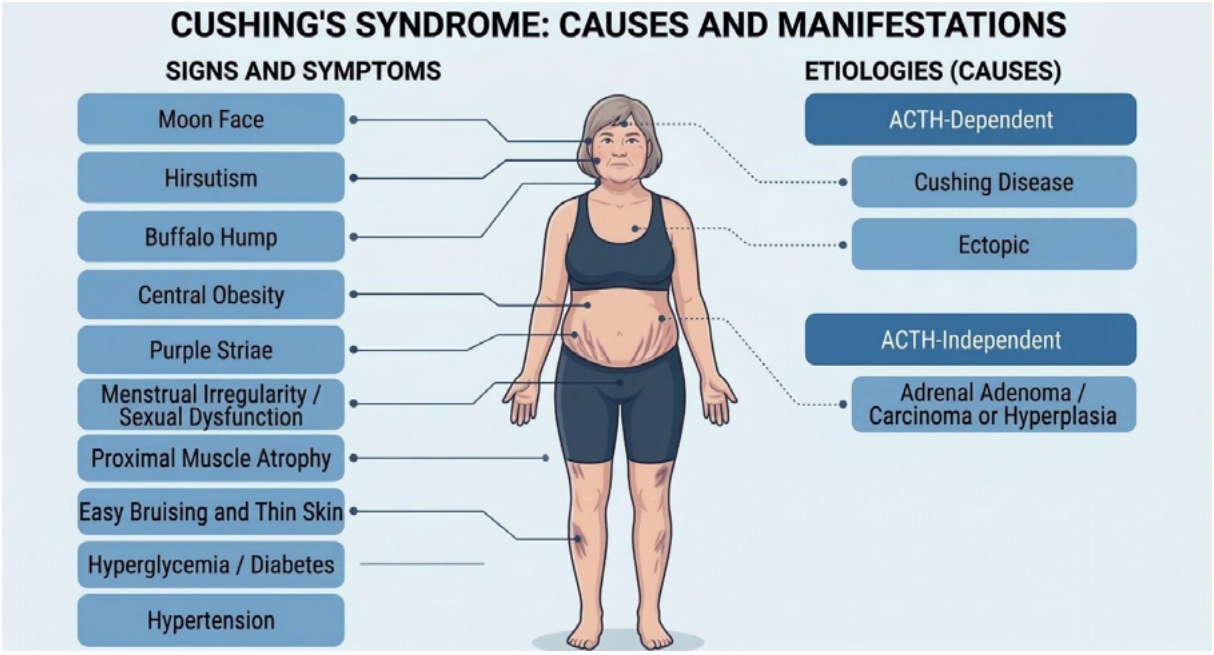
Therefore, biochemical investigation should be pursued in patients with suggestive clinical findings. Prior to biochemical evaluation, exogenous glucocorticoid use must be excluded, as it remains the most frequent cause of hypercortisolism; this includes oral, injectable, inhaled, intra-articular, topical, and ophthalmic formulations[1].

Laboratory Evaluation

Laboratory evaluation represents the next step following a thorough medical history and complete physical examination. A history of obesity, depression, and alcohol use is particularly relevant in differentiating true Cushing's syndrome from pseudo-Cushing states[3]. The diagnosis of Cushing's syndrome relies primarily on biochemical confirmation of hypercortisolism and assessment of cortisol secretion patterns. Exogenous glucocorticoid use — whether oral, injectable, inhaled, intra-articular, or topical, including cutaneous and hair-care formulations — must be carefully excluded[4]. Additionally, medications that increase corticosteroid-binding globulin (CBG) levels or induce CYP3A4 activity may yield false-positive results. Cyclical Cushing's syndrome may further complicate the interpretation of biochemical findings[4]. Notably, the "hook effect" — a well-recognized immunoassay artifact in which markedly elevated hormone concentrations paradoxically produce falsely low results due to antibody saturation — has not been reported in corticotroph adenomas, as these tumors rarely attain sufficient size to generate exceptionally high hormone concentrations[4,5]. These considerations underscore the importance of performing three established first-line screening tests for the accurate diagnosis of Cushing's syndrome, which remains a complex and diagnostically challenging condition[3–6].

- **Late-night salivary cortisol (LNSC):** a minimum of two measurements is recommended, particularly when the initial result is inconclusive.

Figure 1. Causes and Manifestations of Cushing's Syndrome.*Adapted from: Nieman,* L.K. et al., 2025. (Ref. No. 6).



- **24-hour urinary free cortisol (UFC):** at least two collections should be obtained in the setting of diagnostic uncertainty.
- **1 mg overnight dexamethasone suppression test (DST).**

These tests evaluate distinct aspects of cortisol physiology and must be interpreted with caution, taking into account their inherent limitations and potential sources of interference[6,7].

Late-Night Salivary Cortisol (LNSC)

Late-night salivary cortisol assesses the loss of the circadian nadir of cortisol secretion, a hallmark feature of hypercortisolism. Multiple measurements are generally recommended to enhance diagnostic accuracy.

Protocol: Two to three samples are collected at the patient's habitual bedtime (approximately midnight).

Clinical utility: This method demonstrates high sensitivity and specificity, and is particularly valuable in the evaluation of cyclic Cushing's syndrome and in longitudinal clinical follow-up.

Limitations: Its use should be avoided in shift workers owing to disruption of the circadian rhythm. Tandem mass spectrometry-based assays demonstrate superior analytical sensitivity relative to immunoassays, albeit with a marginal reduction in specificity[2,3,8].

24 Hour Urinary Free Cortisol(UFC)

Twenty-four-hour urinary free cortisol quantifies global hypercortisolism by measuring the excretion of biologically active cortisol over a complete 24-hour period.

Protocol: Two to three collections are recommended owing to intraindividual variability in cortisol secretion.

Advantages: This method is independent of corticosteroid-binding globulin (CBG) fluctuations and does not require synchronization with medication administration.

Limitations: UFC demonstrates lower sensitivity relative to the dexamethasone suppression test (DST) and late-night salivary cortisol (LNSC). Its use is not recommended in patients with significant renal impairment (creatinine clearance <60 mL/min) or polyuria (>5 L/day), as these conditions may directly interfere with urinary cortisol excretion[5,7].

Dexamethasone Suppression Test (DST)

The dexamethasone suppression test evaluates the integrity of the hypothalamic- pituitary-adrenal (HPA) axis glucocorticoid negative feedback mechanism. In healthy individuals, dexamethasone suppresses adrenocorticotropic hormone (ACTH) secretion and consequently reduces serum cortisol levels. In patients with Cushing's syndrome, this suppression is characteristically inadequate[8,9].

Protocol: A single oral dose of 1 mg dexamethasone is administered between 11 PM and midnight, with serum cortisol measurement performed at 8 AM the following morning.

Reference value: A serum cortisol level below 1.8 µg/dL (50 nmol/L) indicates a normal suppression response and confers a high negative predictive value for Cushing's syndrome. Values exceeding 3–5 µg/dL are more indicative of overt hypercortisolism, particularly in the context of adrenal incidentalomas[5,7,8].

Advantages: The DST demonstrates high sensitivity and is regarded as the most sensitive first-line screening test for Cushing's syndrome.

Limitations:

- False-positive results may arise from dexamethasone malabsorption, concomitant use of CYP3A4 enzyme inducers (e.g., phenobarbital, carbamazepine), or conditions associated with elevated corticosteroid-binding globulin (CBG) levels, such as pregnancy and exogenous estrogen therapy.
- False-negative results may occur with the use of dexamethasone metabolism inhibitors (e.g., fluoxetine, diltiazem) or in states of reduced CBG levels (e.g., nephrotic syndrome). Diagnostic sensitivity is further reduced in obese patients and those with type 2 diabetes mellitus[9,10].

All first-line diagnostic tests for Cushing's syndrome demonstrate high sensitivity and specificity. Among these, the DST yields the highest sensitivity, whereas 24-hour UFC exhibits comparatively lower sensitivity. The specificity of all first-line screening tests appears to be broadly comparable[8]. Notably, late-night salivary cortisol (LNSC) represents the diagnostic test of choice in patients with suspected Cushing's disease, particularly in the presence of chronic kidney disease (CKD)[11].

TREATMENT

Transsphenoidal resection of the ACTH-secreting pituitary adenoma represents the gold-standard treatment for Cushing's disease. This approach achieves

biochemical remission and resolution of hypercortisolism in the majority of patients. The endoscopic endonasal approach is widely employed, affording superior surgical visualization and reduced postoperative morbidity. Surgical outcomes are closely dependent on the expertise of the multidisciplinary team, with complete tumor resection constituting the primary determinant of remission[2,14].

MEDICAL THERAPY

Medical therapy is indicated for patients who are not surgical candidates, present with persistent or recurrent disease, or require preoperative cortisol control[11]. The primary therapeutic goal is to normalize cortisol levels, ameliorate metabolic complications, and reduce overall disease burden. Combination ther-

Tabela 2. Pharmacological Treatment of Cushing's Disease.

Class / Drug	Mechanism of Action	Administration	Clinical Consideration	Main Adverse Effects
Esteroidogenesis Inhibitors				
Metyrapone	Inhibits 11β-hydroxylase (↓ cortisol)	Oral	Rapid action; useful in severe hypercortisolism; may worsen hyperandrogenism	Gastrointestinal (GI) Symptoms, hirsutism, hypertension, hypokalemia, adrenal insufficiency
Osilodrostat	Inhibits 11β-hydroxylase	Oral	Potent; requires monitoring of cortisol and QTc	Nausea, headache, adrenal insufficiency, ↑ QTc
Cetoconazol	Inhibits multiple CYP450	Oral	Risk of hepatotoxicity; avoid in liver disease	Hepatotoxicity, male hypogonadism, ↑transaminases, ↑ QTc
Levocetoconazol	Inhibits steroidogenesis (CYP3A4)	Oral	Possibly lower hepatotoxicity risk; monitor liver function	GI symptoms, ↑ transaminases, possible ↑ QTc
Mitotano	Direct adrenolytic action	Oral	Slow onset, requires therapeutic drug monitoring; induces adrenal insufficiency	GI symptoms, CNS effects, dyslipidemia, teratogenicity
Etomidato	Inhibits 11β-hydroxylase	Intravenous	Indicated in emergencies; restricted to intensive care settings	Sedation, requires continuous monitoring
ACTH Secretion Inhibitors				
Cabergoline	Dopamine agonist (↓ ACTH)	Oral	Best response in mild cases; possible loss of efficacy over time	GI symptoms, dizziness, impulse control disorders
Pasireotide	Somatostatin analogue	Subcutaneous	May worsen glycemic control; requires monitoring	Hyperglycemia, diarrhea, nausea, cholelithiasis
Pasireotide LAR	Somatostatin analog (long- acting)	Intramuscular	Better adherence (monthly use); similar metabolic effects	Similar to standard formulation
Antagonista do receptor de glicocorticoide				
Mifepristone	Blocks glucocorticoid receptor	Oral	Useful in glycemic control dysregulation; clinical monitoring required (cortisol level unreliable)	Hypokalemia, edema, endometrial thickening, nausea

Adapted from:Fleseriu M,et al., 2021(Ref.Nº1), Nieman, L.K. et al., 2025.(Ref.Nº 6).

apy may be employed when monotherapy proves insufficient[1,16,17]. Additional therapeutic modalities include radiotherapy — which is characterized by a slow onset of action — and bilateral adrenalectomy, the latter providing rapid cortisol control but necessitating lifelong hormonal replacement therapy and carrying a risk of corticotroph tumor progression (Nelson's syndrome)[3,17,18.]

REMISSION AND RECURRENCE

Remission is defined by recovery of the hypothalamic-pituitary-adrenal (HPA) axis following surgical intervention, with biochemical criteria established as a serum cortisol level below 5 µg/dL within the first postoperative week, although some literature advocates a stricter threshold of less than 1.8 µg/dL[8,5,19]. Recurrence may manifest as a late event, with progressive elevation of cortisol levels over weeks to months, accompanied by the reappearance of clinical features — including weight gain, insomnia, and arterial hypertension — alongside biochemical alterations. Late-night salivary cortisol (LNSC) demonstrates superior sensitivity relative to 24-hour urinary free cortisol (UFC) for the early detection of recurrence. Even following confirmed remission, previously acquired comorbidities may persist and warrant continued clinical surveillance[20].

COMPLICATIONS AND COMORBIDITIES

Cushing syndrome is associated with multiple systemic complications that significantly contribute to increased morbidity and mortality. A hypercoagulable state is commonly observed, leading to an elevated risk of venous thromboembolism. Additionally, patients exhibit increased cardiovascular risk, often in association with obesity, type 2 diabetes mellitus, and dyslipidemia[5,21].

In the musculoskeletal system, patients may develop metabolic bone disease characterized by reduced bone mineral density and an increased incidence of fractures, particularly vertebral fractures. Growth hormone (GH) deficiency is relatively common following treatment, especially in cases of central etiology. GH replacement therapy may offer benefits such as improved body composition, increased bone mineral density, and enhanced quality of life; however, it may adversely affect glycemic control[9,10,22].

DIFFERENTIATION BETWEEN PITUITARY AND ECTOPIC SOURCES

When the source of adrenocorticotropic hormone (ACTH) excess remains indeterminate, inferior petrosal sinus sampling (IPSS) is regarded as the gold standard for etiological differentiation. Upon confirmation of ectopic ACTH secretion, further diagnostic evaluation should include thoracic computed tomography (CT), abdominal CT or magnetic resonance imaging (MRI), and positron emission tomography– computed tomography (PET-CT) in selected cases. The neoplasms most commonly implicated in this context comprise small-cell lung carcinoma, bronchial neuroendocrine tumors, and pancreatic neuroendocrine tumors[22,23].

CONCLUSION

This case report underscores the importance of maintaining a high index of clinical suspicion for Cushing's syndrome, particularly Cushing's disease, in patients presenting with difficult-to-control metabolic syndrome. Although conditions such as type 2 diabetes mellitus, obesity, hypertension, and dyslipidemia are highly prevalent, cases characterized by treatment resistance and rapid progression of target-organ damage should prompt consideration of secondary etiologies.

In this context, a comprehensive clinical assessment, combined with strict adherence to a structured diagnostic algorithm, is essential for accurate diagnosis. The systematic approach to the investigative process—from initial screening tests to definitive etiological confirmation of adrenocorticotropic hormone (ACTH)-dependent hypercortisolism—was crucial in elucidating the present case.

Despite its rarity, Cushing's disease carries substantial clinical significance and poses considerable management challenges. Early diagnosis, timely implementation of appropriate therapeutic interventions, surgical management when indicated, and individualized long-term follow-up are critical to reducing morbidity and improving patient quality of life.

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